FIGURE 1A

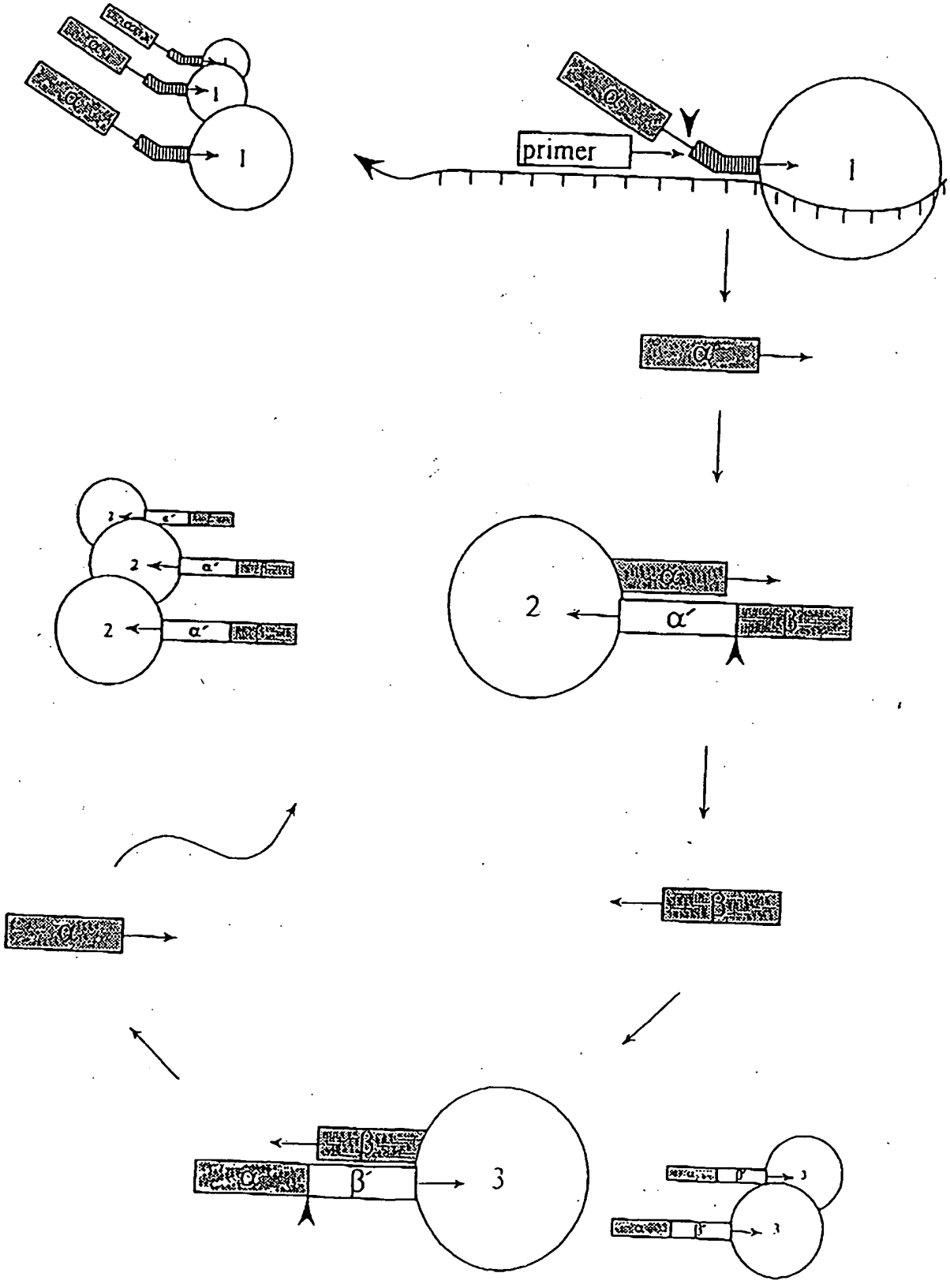
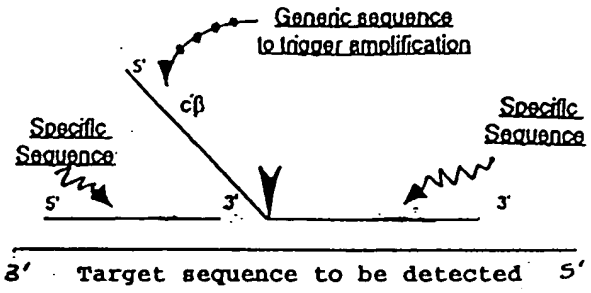
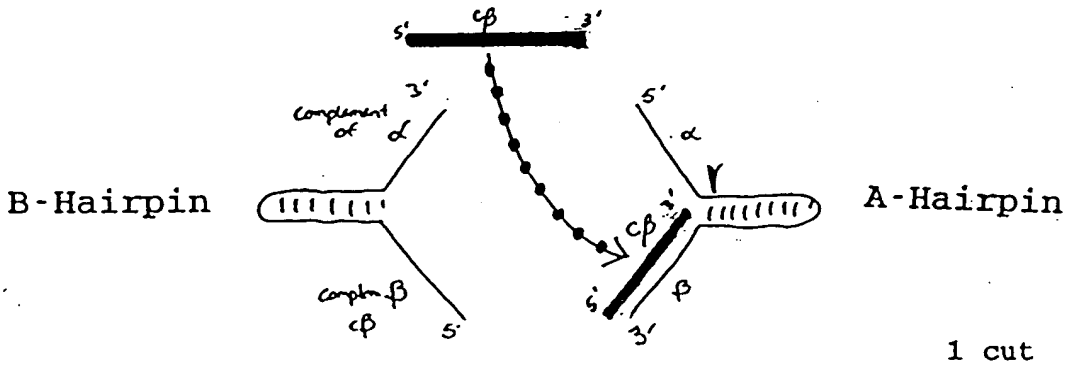


FIGURE 1 B

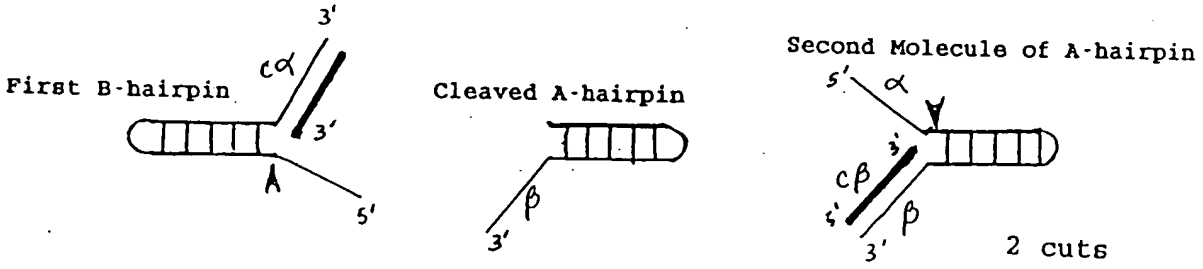
PART ONE: TRIGGER REACTION



PART TWO: DETECTION REACTION



Denature, anneal



Denature, anneal

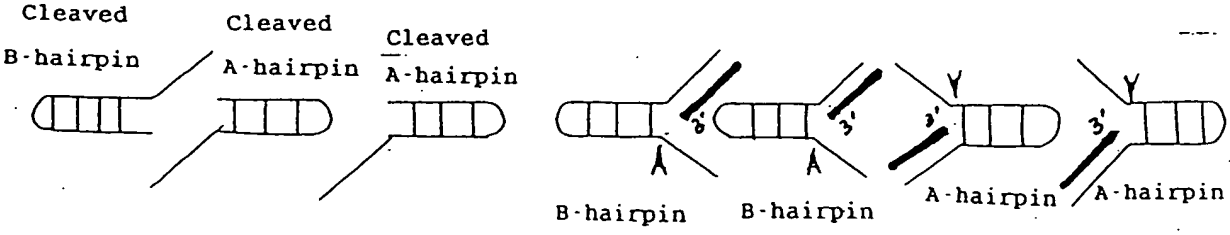


FIGURE 2

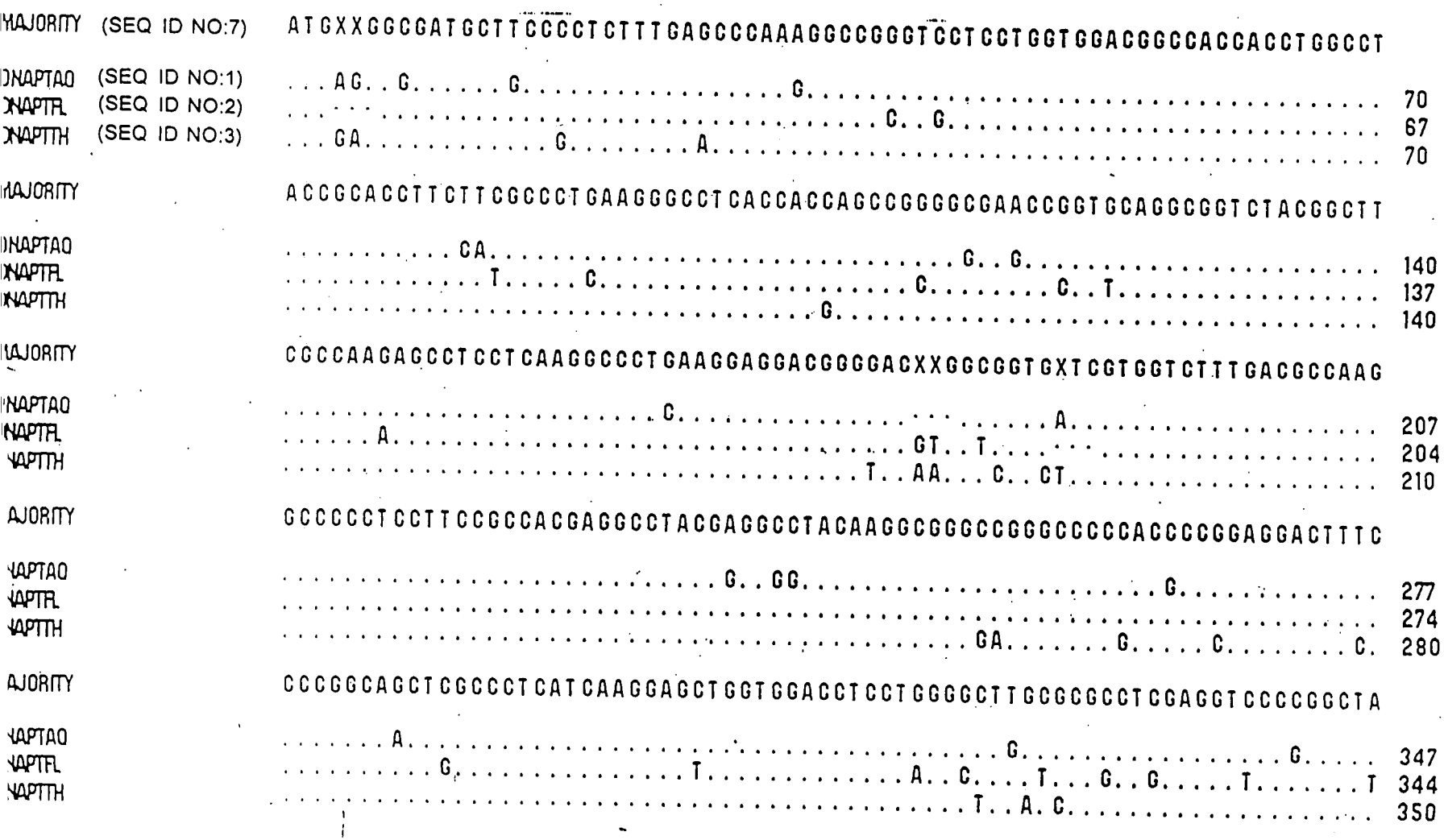

MAJORITY (SEQ ID NO:7) ATGXXGGCGATGCTTCCCCTCTTTGAGCCCAAAGGCCGGGTCCTCCTGGTGGACGGCCACCACCTGGCCT
)NAPTAQ (SEQ ID NO:1) ...AG..G.....G.....G..... 70
)NAPTFL (SEQ ID NO:2) .....C..G..... 67
)NAPTTH (SEQ ID NO:3) ...GA.....G.....A..... 70
MAJORITY ACCGCACCTTCTTCGCCCTGAAGGGCCTCACCACCAGCCGGGGCGAACCGGTGCAGGCGGTCTACGGCTT
)NAPTAQ .....CA.....G..G..... 140
)NAPTFL .....T.....C.....C.....C..T..... 137
)NAPTTH .....G..... 140
MAJORITY CGCCAAGAGCCTCCTCAAGGCCCTGAAGGAGGACGGGGACXXGGCGGTGXTCGTGGTCTTTGACGCCAAG
)NAPTAQ .....C.....A..... 207
)NAPTFL .....A.....GT..T..... 204
)APTTH .....T..AA...C..CT..... 210
AJORITY GCCCCCTCCTTCCGCCACGAGGCCTACGAGGCCTACAAGGCGGGCCGGGCCCCCACCCCGGAGGACTTTC
)APTAQ .....G..GG.....G..... 277
)APTFL 274
)APTTH .....GA.....G.....C.....C. 280
AJORITY CCCGGCAGCTCGCCCTCATCAAGGAGCTGGTGGACCTCCTGGGGCTTGCGCGCCTCGAGGTCCCCGGCTA
)APTAQ .....A.....G.....G..... 347
)APTFL .....G.....T.....A..C....T...G..G.....T.....T 344
)APTTH .....T..A.C..... 350

FIGURE 2 (cont'd)

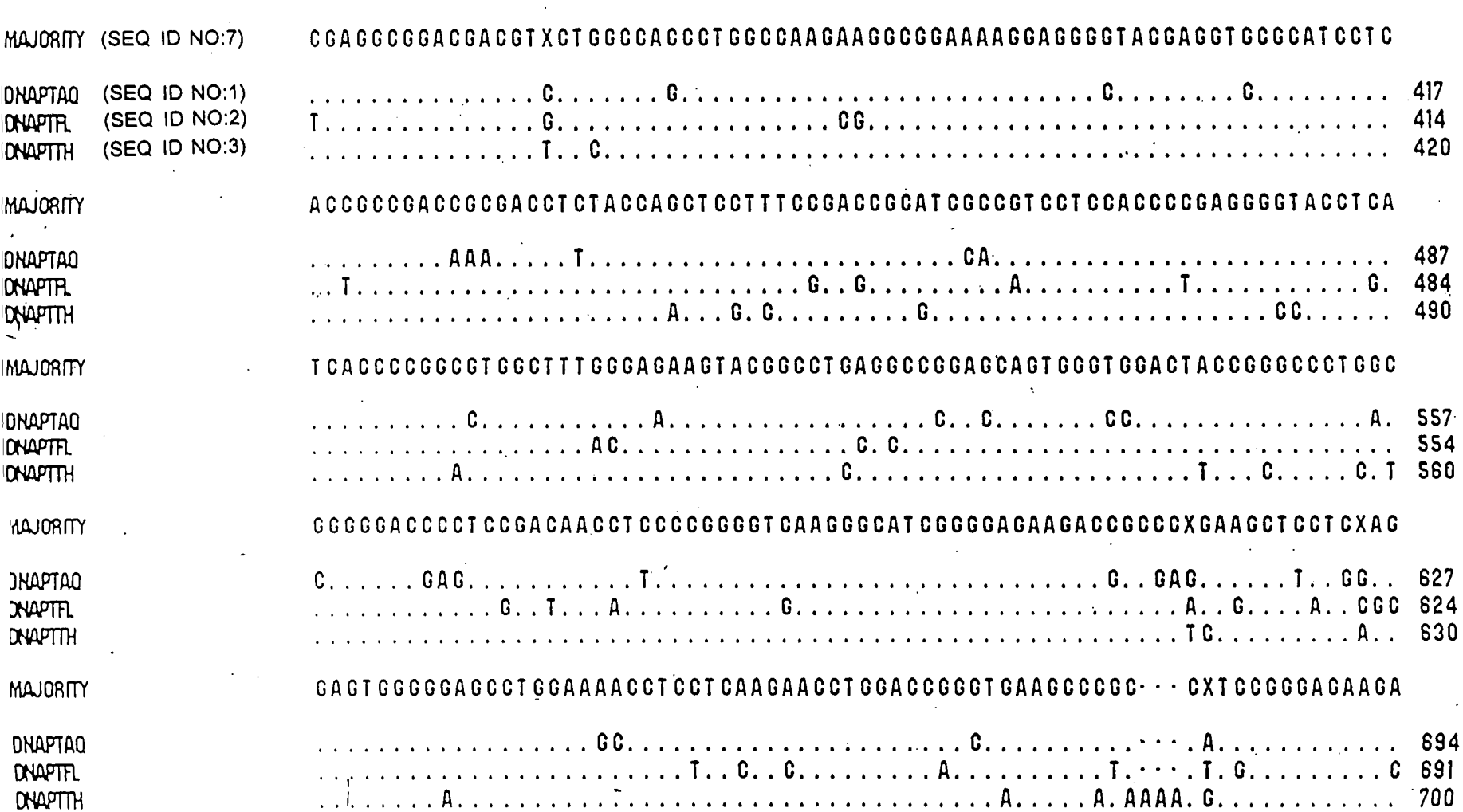


FIGURE 2 (cont'd)

```
AJORITY (SEQ ID NO:7)  TCCAGGCCCACATGGAXGACCTGAXGCTCTCCTGGGAGCTXTCCCAGGTGCGCACCGACCTGCCCCTGGA

NAPTAQ  (SEQ ID NO:1)  ...T..........C..T....A..........C..GG..A.....................  764
NAPTFL  (SEQ ID NO:2)  .......GGG.....G.C....GCC..T...C..A....T.........A...T.....  761
NAPTTH  (SEQ ID NO:3)  ..A........C....A.....C.G........T......C....G..............C.....  770

AJORITY                GGTGGACTTCGCCAAGXGGCGGGAGCCCGACCGGGAGGGCCTTAGGGCCTTTCTGGAGAGGCTGGAGTTT

NAPTAQ                 ..............AA.....................A.........................T.....  834
NAPTFL                 ..........GG.G.C.C..CACA...A...T.....T..GC....T...T.....C..T.....  831
NAPTTH                 .......C.....C..G.....................................C..............C  840

AJORITY                GGCAGCCTCCTCCACGAGTTCGGCCTCCTGGAGGGCCCCAAGGCCCTGGAGGAGGCCCCCTGGCCCCCGC

NAPTAQ                 ........................T.....AA.....................................  904
NAPTFL                 ..A.....................................G..G.....GGCA.....................T.  901
NAPTTH                 .....................................C....GCCC.....................  910

AJORITY                CGGAAGGGGCCTTCGTGGGCTTTGTCCTTTCCCGCCCCGAGCCCATGTGGGCCGAGCTTCTGGCCCTGGC

NAPTAQ                 ........................G.........AAG.................T.............  974
NAPTFL                 ..........T..TT........TC.T.......T.....................................  971
NAPTTH                 ......................C.....C.....................G.....AAA.....  980

AJORITY                CGCCGCCAGGGAGGGCCGGGTCCACCGGGCACCAGACCCCTTTAXGGGCCTXAGGGACCTXAAGGAGGTG

NAPTAQ                 ..........G.....................C..C..G..T.A..AA.C...C........G.......C.  1044
NAPTFL                 T.GG...GT.......G..CC....T.......A.......C...G......G........T....G....  1041
NAPTTH                 ....TG......C.........G.........G..........GGC...G..A.A......C........C  1050
```

FIGURE 2 (cont'd)

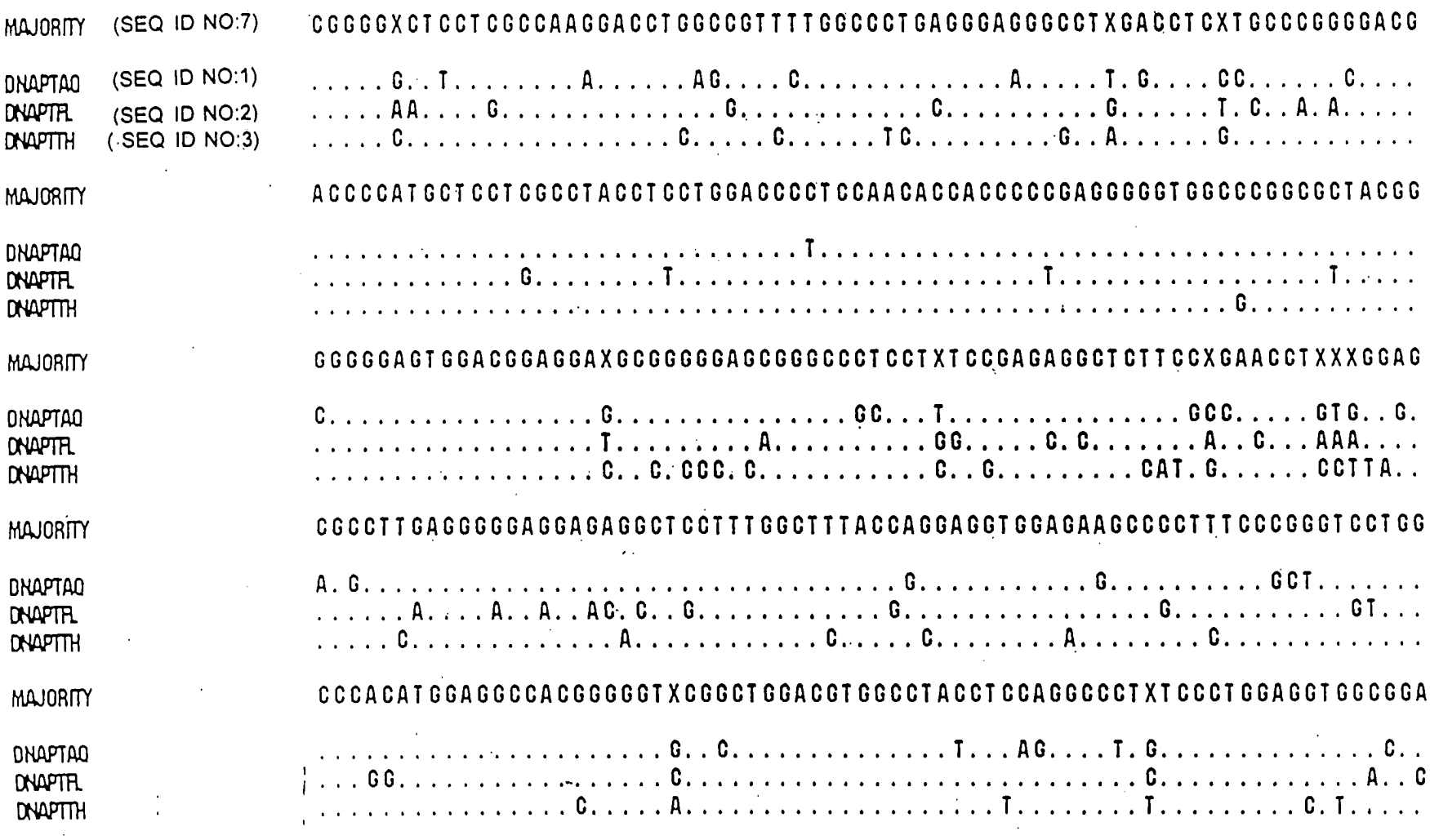


FIGURE 2 (cont'd)

```
MAJORITY (SEQ ID NO:7)  GGAGATCCGCCGCCTCGAGGAGGAGGTCTTCCGCCTGGCCGGCCACCCCTTCAACCTCAACTCCCGGGAC

DNAPTAQ  (SEQ ID NO:1)  .......GC..........CC.....................................................
DNAPTFL  ( SEQ ID NO:2) ....G.G....AG..G.....................................................C...
DNAPTTH  (SEQ ID NO:3)  ..................................T....G.....................................

MAJORITY                CAGCTGGAAAGGGTGCTCTTTGACGAGCTXGGGCTTCCCGCCATCGGCAAGACGGAGAAGACXGGCAAGC

DNAPTAQ                 ..............C..............A.....................................C.......
DNAPTFL                 ........GC.....................G..C..G..T.....................G..G..A.
DNAPTTH                 ..........................TA..........T.G..G......C.A.....A.......

MAJORITY                GCTCCACCAGCGCCGCCGTGCTGGAGGCCCTXCGXGAGGCCCACCCCATCGTGGAGAAGATCCTGCAGTA

DNAPTAQ                 ..................C..........C..C.....................................
DNAPTFL                 ............T.................G..A.....................CCGC.....
DNAPTTH                 ...............G.............A..G.....................................C...C.

MAJORITY                CCGGGAGCTCACCAAGCTCAAGAACACCTACATXGACCCCCTGCCXGXCCTCGTCCACCCCAGGACGGGC

DNAPTAQ                 ................G....G.........T......T....G.A....A.....................
DNAPTFL                 ..................................A..........C.C...G.........A...C...
DNAPTTH                 ..............................G.G........C..AAG...........G.........

MAJORITY                CGCCTCCACACCCGCTTCAACCAGACGGCCACGGCCACGGGCAGGCTTAGTAGCTCCGACCCCAACCTGC

DNAPTAQ                 ..................................................A..........T........C.
DNAPTFL                 ..G.............................C..............TCC.....................
DNAPTTH                 ..........................................G.....................
```

FIGURE 2 (cont'd)

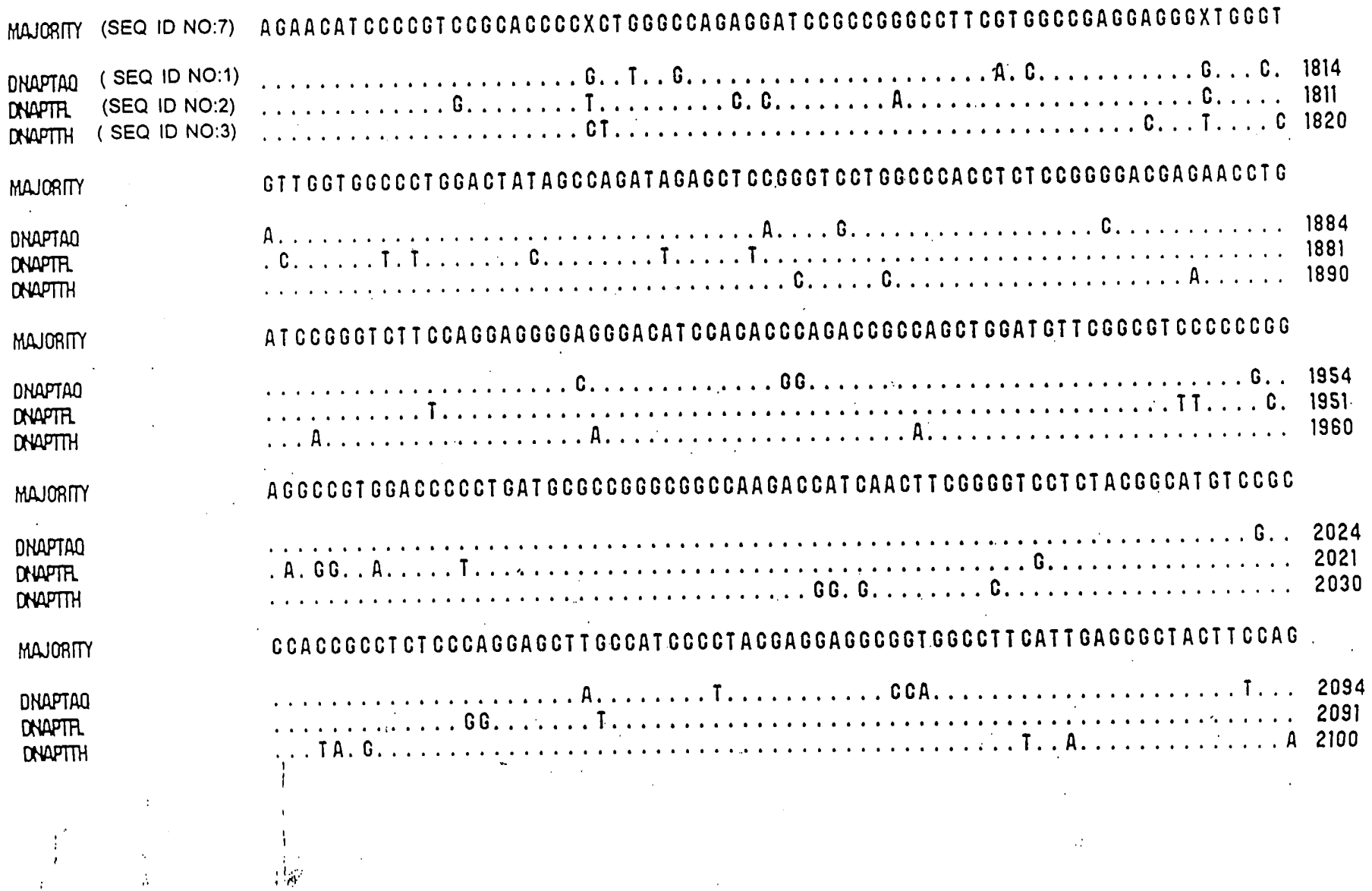


FIGURE 2 (cont'd)

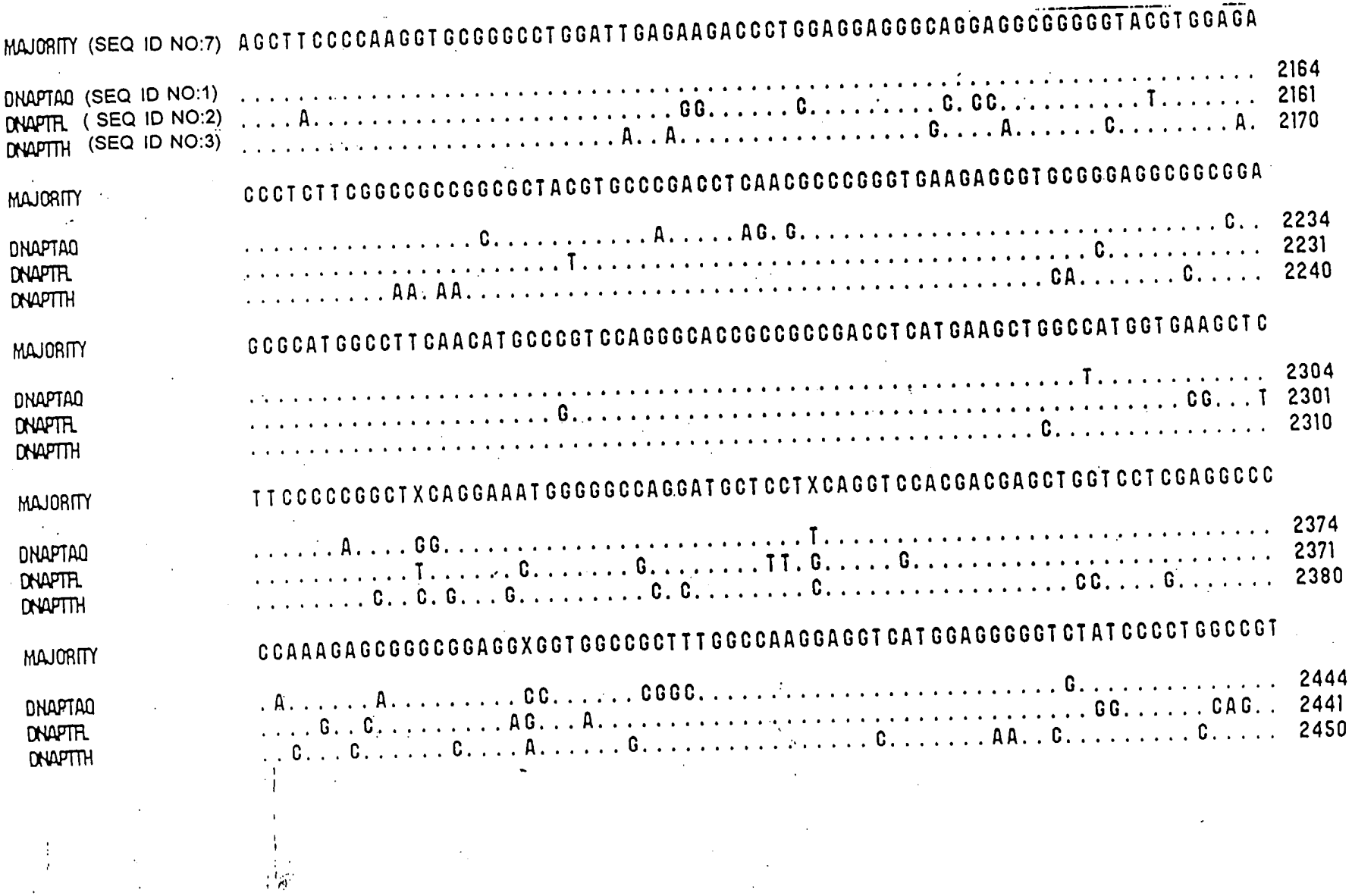


FIGURE 2 (cont'd)

```
MAJORITY  (SEQ ID NO:7)  GCCCCTGGAGGTGGAGGTGGGGATGGGGGAGGACTGGCTCTCCGCCAAGGAGTAG

DNAPTAQ   (SEQ ID NO:1)  .....A.....GA   2499
DNAPTFL   (SEQ ID NO:2)  .....CC.....   2496
DNAPTTH   (SEQ ID NO:3)  .....T.....GT...   2505
```

FIGURE 3

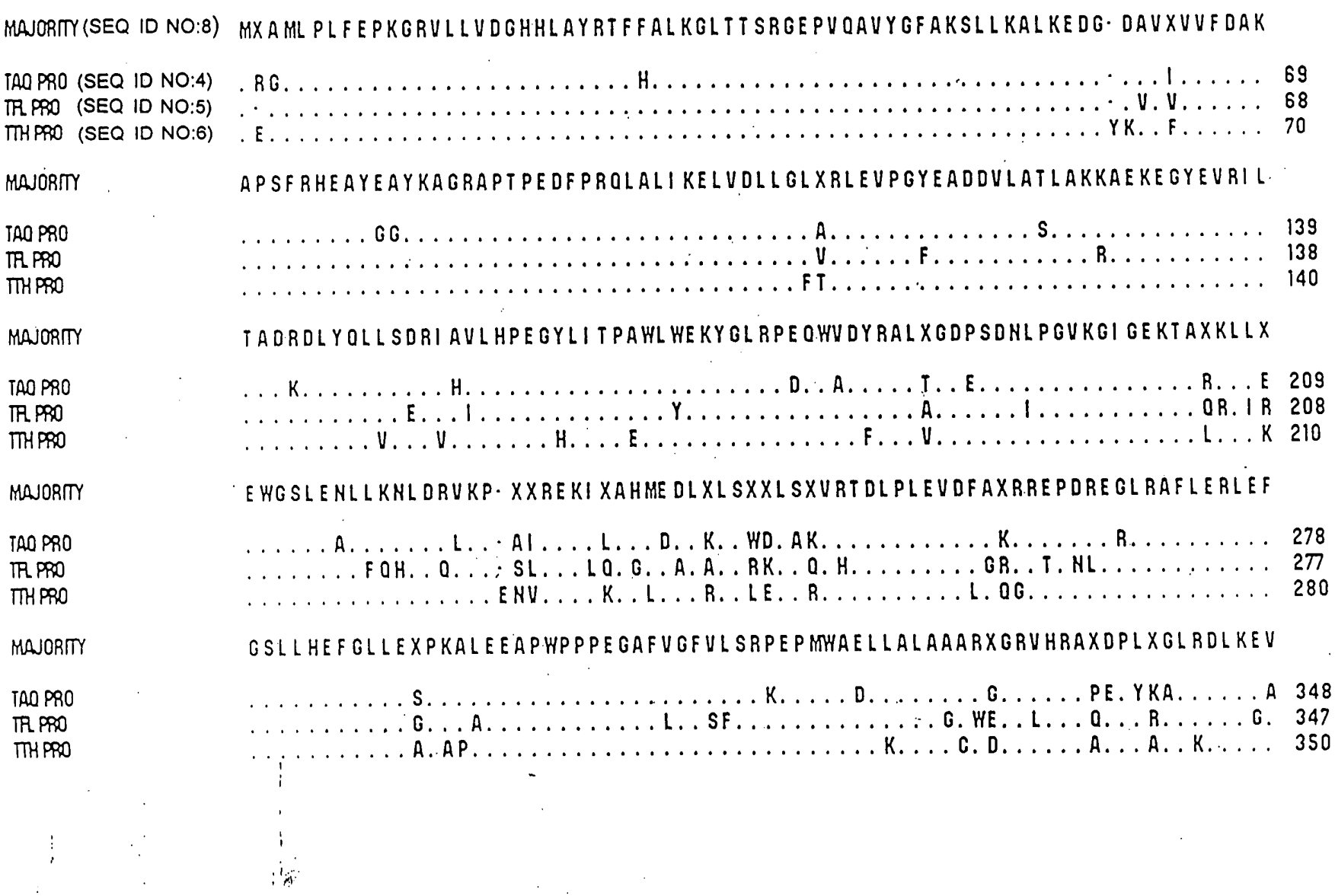

MAJORITY (SEQ ID NO:8) MXAML PLFEPKGRVLLVDGHHLAYRTFFALKGLTTSRGEPVQAVYGFAKSLLKALKEDG· DAVXVVFDAK
TAQ PRO (SEQ ID NO:4) . RG. . . . . H. . . . . I. 69
TFL PRO (SEQ ID NO:5) . . . . . V. V. 68
TTH PRO (SEQ ID NO:6) . E. . . . . YK. . F. 70
MAJORITY APSFRHEAYEAYKAGRAPTPEDFPRQLALIKELVDLLGLXRLEVPGYEADDVLATLAKKAEKEGYEVRIL
TAQ PRO . . . . . GG. . . . . A. . . . . S. 139
TFL PRO . . . . . V. . . . . F. . . . . R. 138
TTH PRO . . . . . FT. 140
MAJORITY TADRDLYQLLSDRIAVLHPEGYLITPAWLWEKYGLRPEQWVDYRALXGDPSDNLPGVKGIGEKTAXKLLX
TAQ PRO . . . K. . . . . H. . . . . D. . A. . . . . T. . E. . . . . R. . . E 209
TFL PRO . . . . . E. . . I. . . . . Y. . . . . A. . . . . I. . . . . QR. I R 208
TTH PRO . . . . . V. . . V. . . . . H. . . . E. . . . . F. . . V. . . . . L. . . K 210
MAJORITY EWGSLENLLKNLDRVKP· XXREKIXAHMEDLXLSXXLSXVRTDLPLEVDFAXRREPDREGLRAFLERLEF
TAQ PRO . . . . . A. . . . . L. . · AI. . . . L. . . D. . K. . WD. AK. . . . . K. . . . . R. 278
TFL PRO . . . . . FQH. . Q. . . · SL. . . LQ. G. . A. A. . RK. . Q. H. . . . . GR. . T. NL. 277
TTH PRO . . . . . ENV. . . . K. . L. . . R. . LE. . R. . . . . L. QG. 280
MAJORITY GSLLHEFGLLEXPKALEEAPWPPPEGAFVGFVLSRPEPMWAELLALAAARXGRVHRAXDPLXGLRDLKEV
TAQ PRO . . . . . S. . . . . K. . . . . D. . . . . G. . . . . PE. YKA. . . . . A 348
TFL PRO . . . . . G. . . A. . . . . L. . SF. . . . . G. WE. . L. . . Q. . . R. . . . . G. 347
TTH PRO . . . . . A. AP. . . . . K. . . . C. D. . . . . A. . . A. . K. 350

FIGURE 3 (cont'd)

```
MAJORITY (SEQ ID NO:8)  RGLLAKDLAVLALREGLDLXPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEDAGERALLSERLFXNLXX

TAQ PRO  (SEQ ID NO:4)  .....S.....G.P.....E.....A.....A..WG  418
TFL PRO  (SEQ ID NO:5)  ..I.....F.E.....A....QT.KE  417
TTH PRO  (SEQ ID NO:6)  .....S.....V.....AH.....HR..LK  420

MAJORITY                RLEGEERLLWLYXEVEKPLSRVLAHMEATGVRLDVAYLQALSLEVAEEIRRLEEEVFRLAGHPFNLNSRD

TAQ PRO                 .....R...R...A.....R.....A...A.....  488
TFL PRO                 ..K.....E.....R.....EA.V.Q.....  487
TTH PRO                 .....K.....H.....L.....  490

MAJORITY                QLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTKLKNTYIDPLPXLVHPRTG

TAQ PRO                 .....S.....D.I.....  558
TFL PRO                 .....DR.....A....K..  557
TTH PRO                 .....R...L...Q.....H.....V....S.....  560

MAJORITY                RLHTRFNQTATATGRLSSSDPNLQNIPVRTPLGQRIRRAFVAEEGWXLVALDYSQIELRVLAHLSGDENL

TAQ PRO                 .....I.....L.....  628
TFL PRO                 .....V..V.....  627
TTH PRO                 .....A..A.....  630

MAJORITY                IRVFQEGRDIHTQTASWMFGVPPEAVDPLMRRAAKTINFGVLYGMSAHRLSQELAIPYEEAVAFIERYFQ

TAQ PRO                 .....E.....R.....Q.....  698
TFL PRO                 .....S..G.....G..S.....  697
TTH PRO                 .....K.....V.....  700
```

FIGURE 3 (cont'd)

```
MAJORITY (SEQ ID NO:8)  SFPKVRAWIEKTLEEGRRRGYVETLFGRRRYVPDLNARVKSVREAAERMAFNMPVQGTAADLMKLAMVKL

TAQ PRO  (SEQ ID NO:4)  ..........................................E.....................................  768
TFL PRO  (SEQ ID NO:5)  .Y........G.....................................................R.  767
TTH PRO  (SEQ ID NO:6)  ..................K.....................................................  770

MAJORITY                FPRLXEMGARMLLQVHDELVLEAPKXRAEXVAALAKEVMEGVYPLAVPLEVEVGXGEDWLSAKEX

TAQ PRO                 ....E..................E...A..R.....................I.........  833
TFL PRO                 ....Q.L................D...R...........W..Q.........L.........  831
TTH PRO                 ....R..............L....QA...E.......A..KA...........M........G  835
```

FIGURE 4

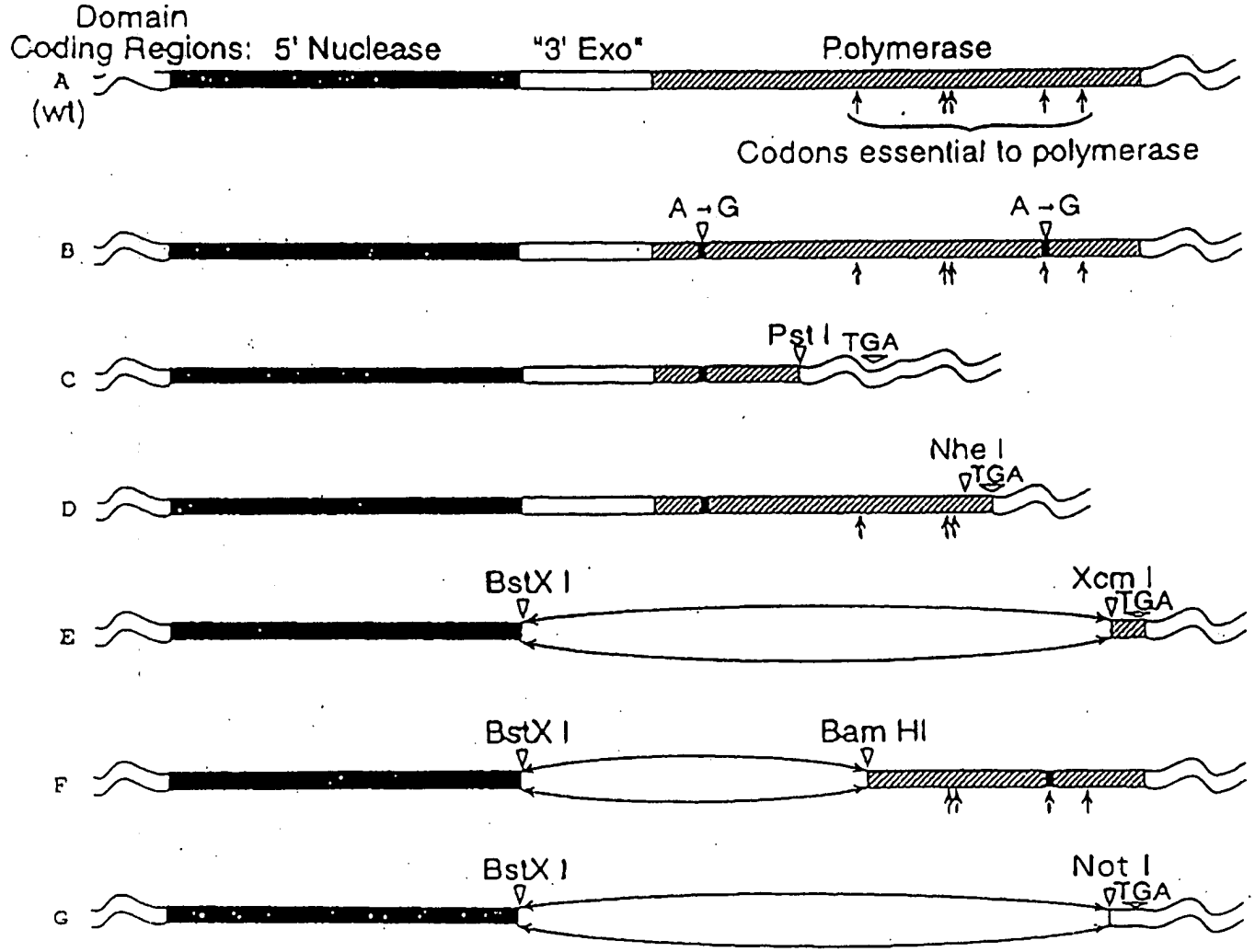

Domain
Coding Regions: 5' Nuclease
"3' Exo"
Polymerase
A
(wt)
Codons essential to polymerase
A → G
A → G
B
Pst I
TGA
C
Nhe I
TGA
D
BstX I
Xcm I
TGA
E
BstX I
Bam HI
F
BstX I
Not I
TGA
G

FIGURE 5

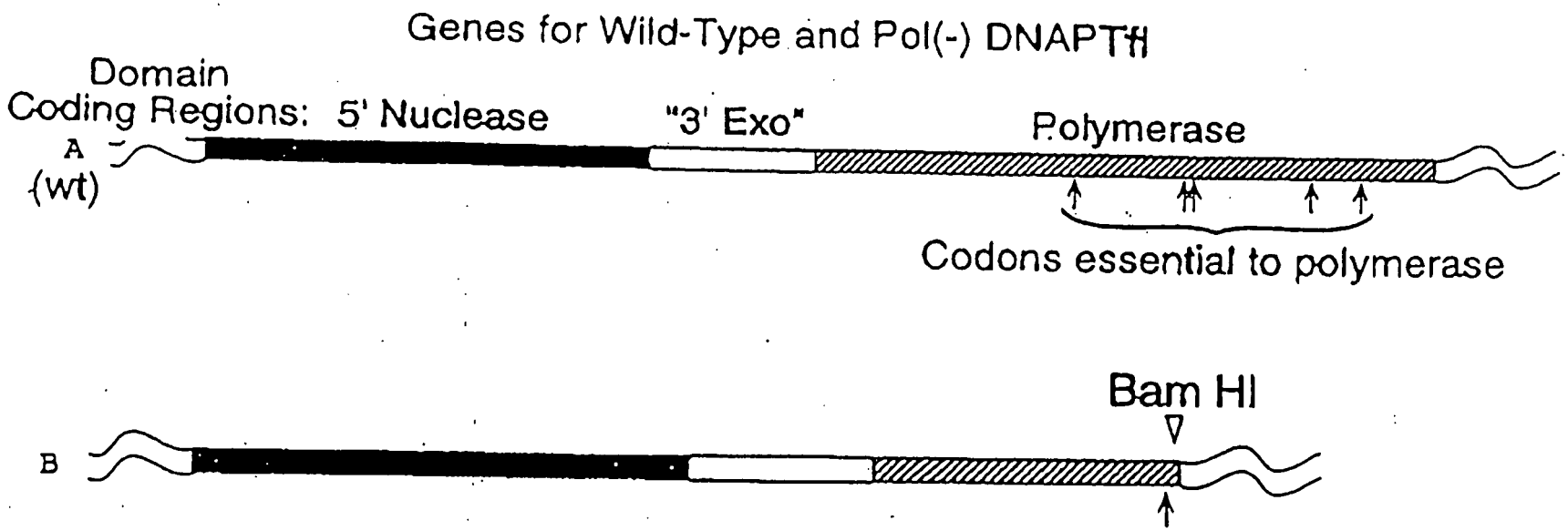

Genes for Wild-Type and Pol(-) DNAPTH
Domain
Coding Regions: 5' Nuclease
"3' Exo"
Polymerase
A
(wt)
Codons essential to polymerase
Bam HI
B

FIGURE 6

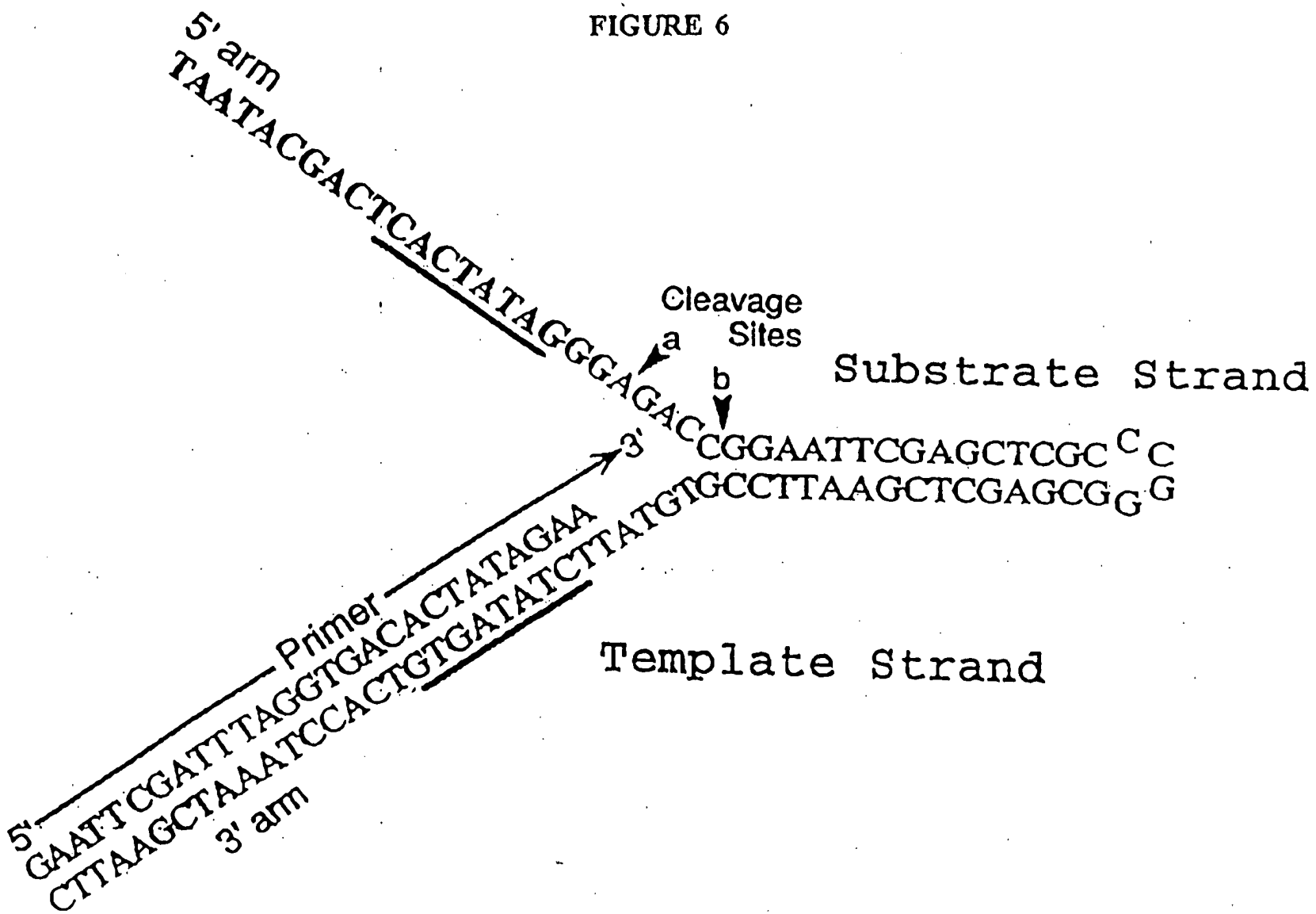

5' arm
TAATACGACTCACTATAGGGAGACC
Cleavage Sites
a
b
Substrate Strand
3'
CGGAATTCGAGCTCGC C C
GCCTTAAGCTCGAGCG G G
Primer
5'
GAATTCGATTTAGGTGACACTATAGAA
CTTAAGCTAAATCCACTGTGATATCTTATGT
3' arm
Template Strand

FIGURE 7

FIGURE 8

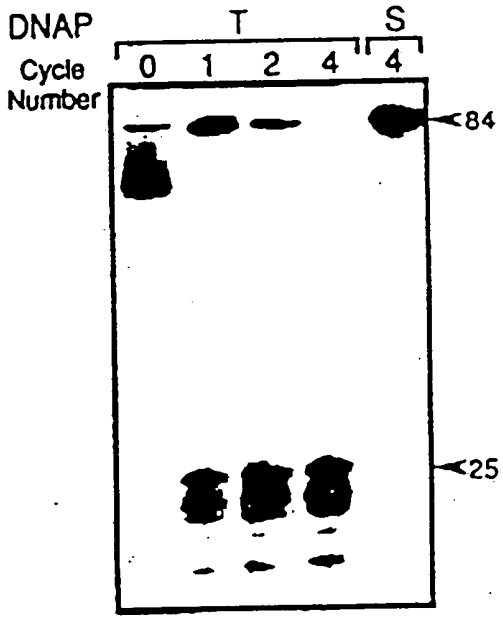


FIGURE 9

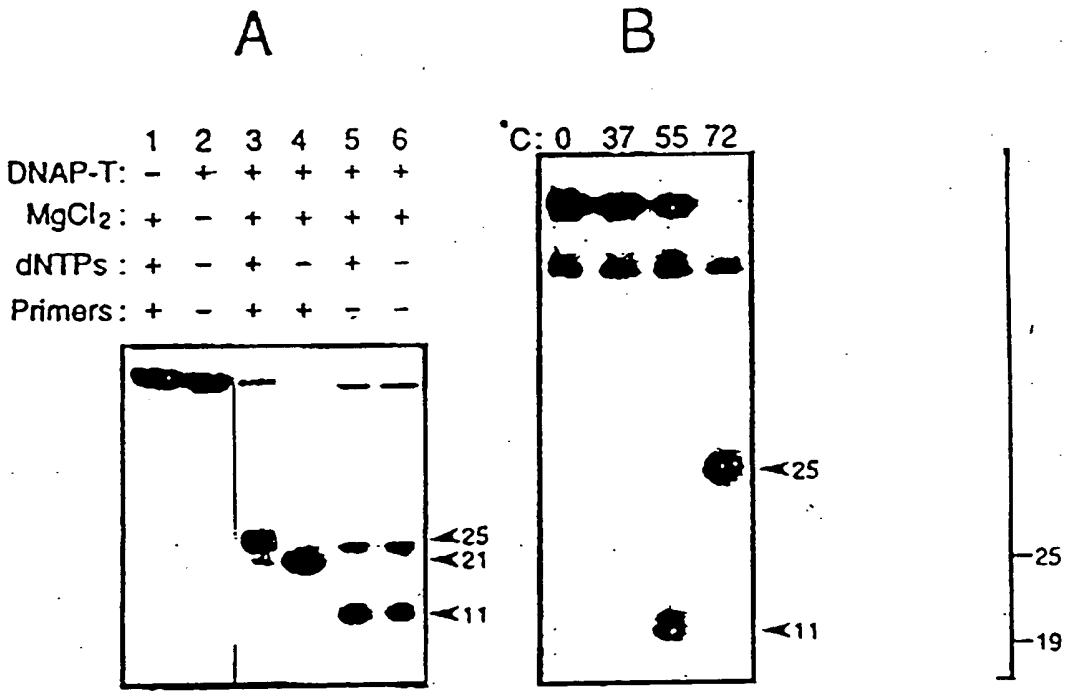

A
B
1 2 3 4 5 6
DNAP-T: – + + + + +
MgCl2: + – + + + +
dNTPs: + – + – + –
Primers: + – + + + –
25
21
11
°C: 0 37 55 72
25
11
25
19

FIGURE 10

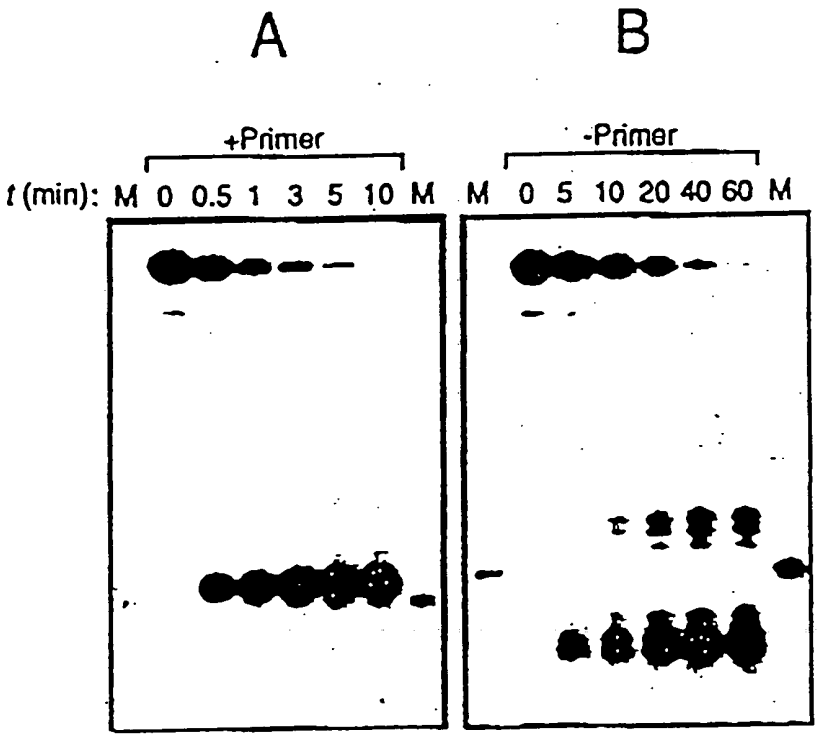


FIGURE 11

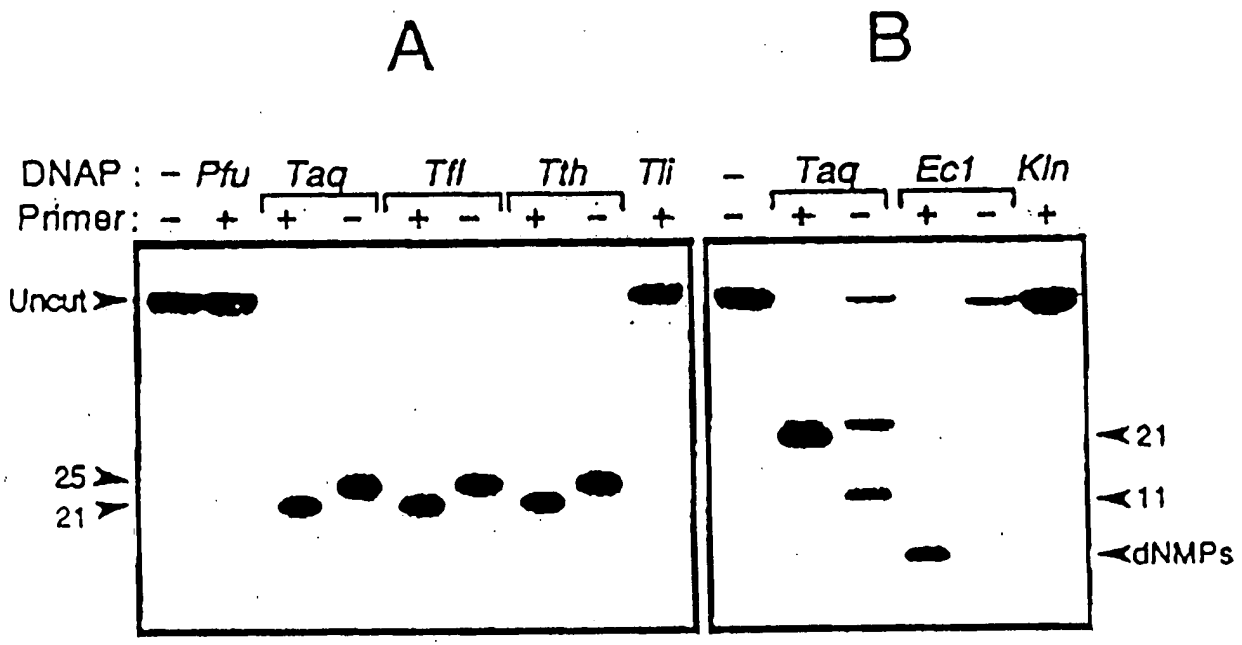

A
B
DNAP : – Pfu Taq Tfl Tth Tli – Taq Ec1 Kln
Primer: – + + – + – + – + – + + – + –
Uncut
25
21
21
11
dNMPs

FIGURE 12

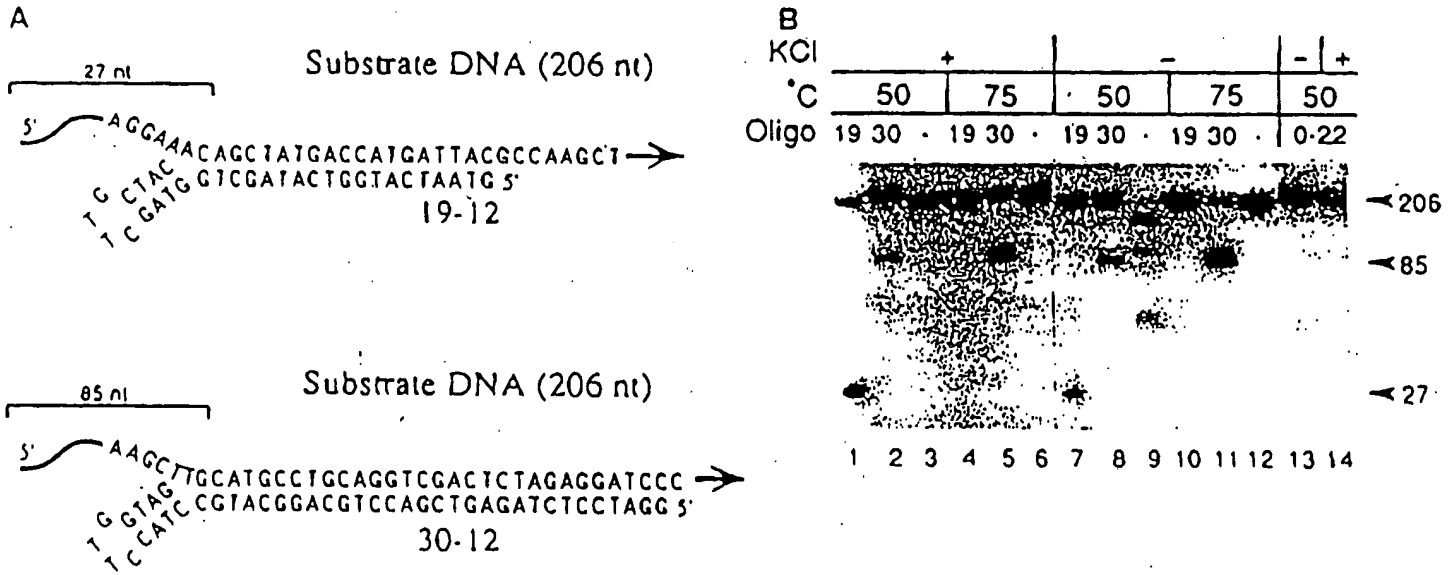

A
27 nt
Substrate DNA (206 nt)
5' AGGAAACAGCTATGACCATGATTACGCCAAGCT
TC CTAC
TC GATG GTCGATACTGGTACTAATG 5'
19-12
85 nt
Substrate DNA (206 nt)
5' AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCC
TG GTAG
TC CATC CGTACGGACGTCCAGCTGAGATCTCCTAGG 5'
30-12
B
KCl + - - +
°C 50 75 50 75 50
Oligo 19 30 · 19 30 · 19 30 · 19 30 · 0·22
206
85
27
1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE 13

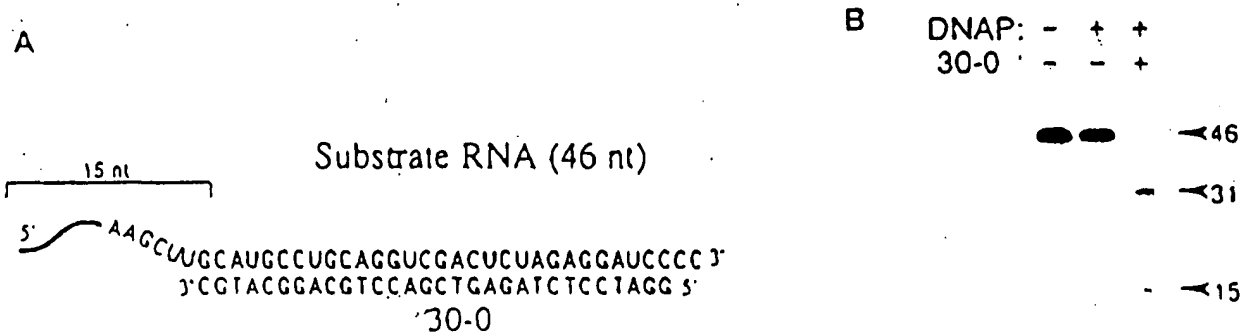
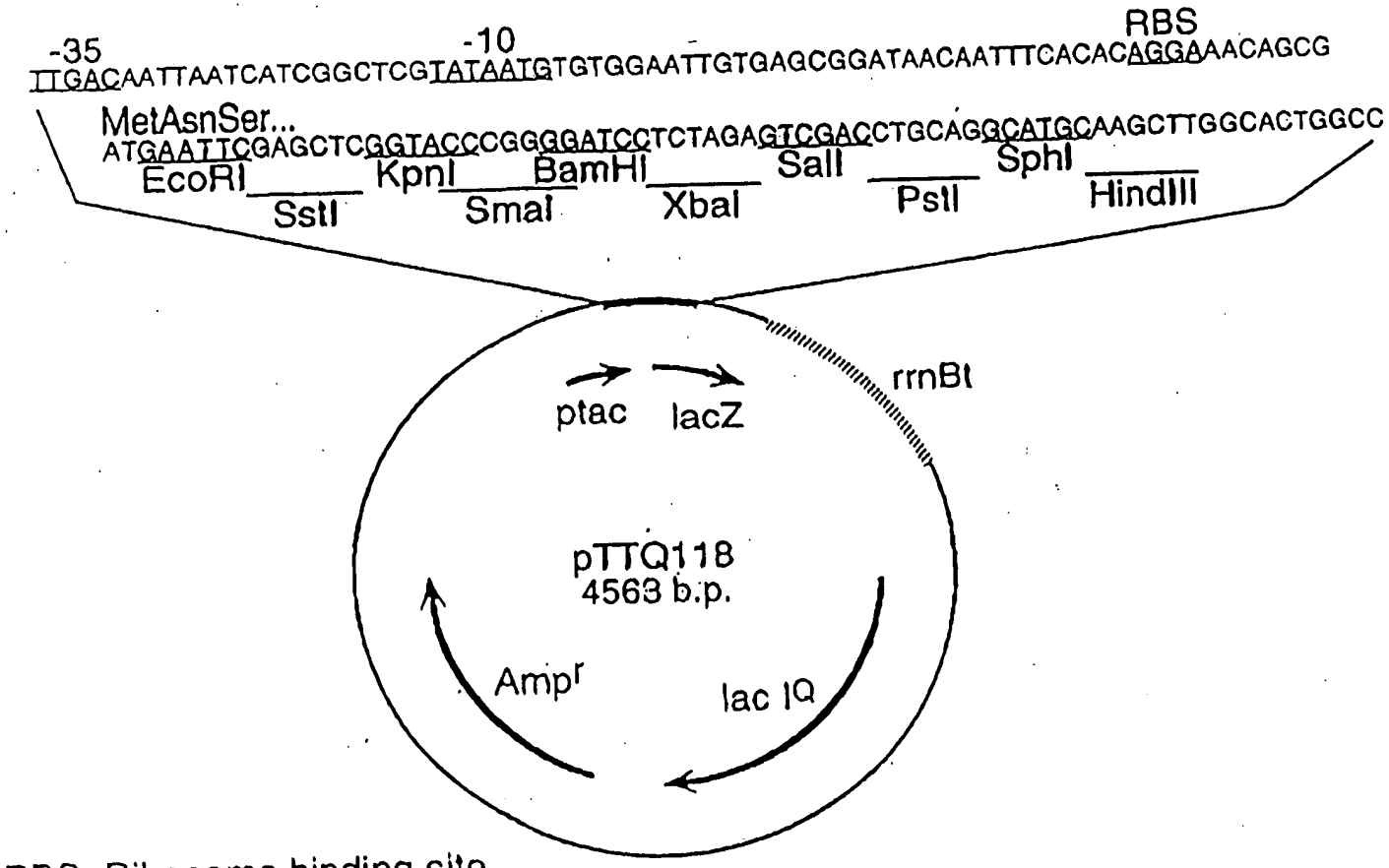

A
Substrate RNA (46 nt)
15 nt
5' AAGCUUGCAUGCCUGCAGGUCGACUCUAGAGGAUCCCC 3'
3' CGTACGGACGTCCAGCTGAGATCTCCTAGG 5'
30-0
B
DNAP: - + +
30-0 - - +
46
31
15

FIGURE 14



RBS: Ribosome binding site

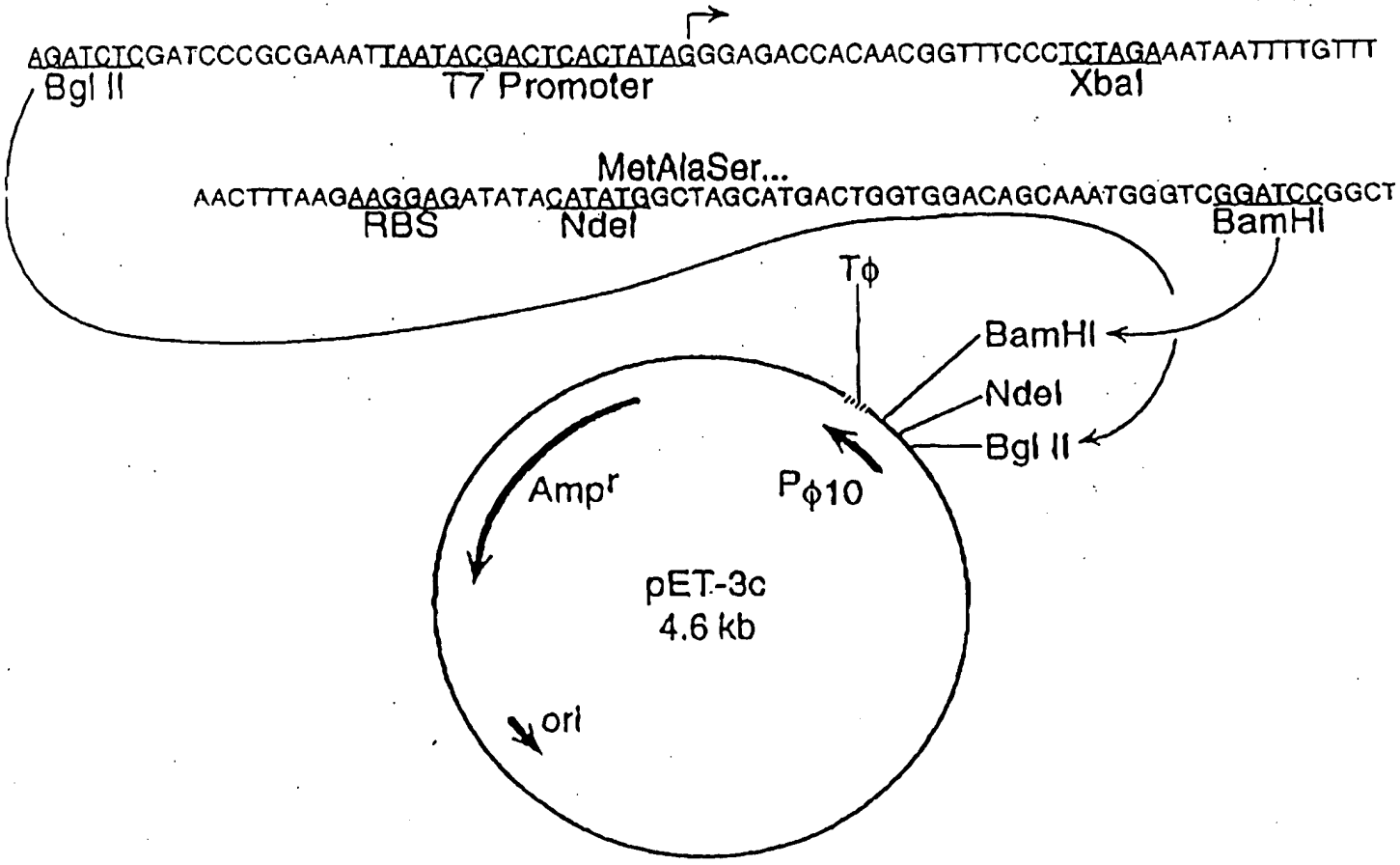
ptac: Synthetic tac promoter

lac I^Q: Lac repressor gene

lacZ: Beta-galactosidase alpha fragment

rrnBt: E. coli rrnB transcription terminator

FIGURE 15



$P_{\phi 10}$: Bacteriophage T7 ϕ10 promoter

RBS: Ribosome binding site

Tϕ: T7 ϕ Terminator

FIGURE 16

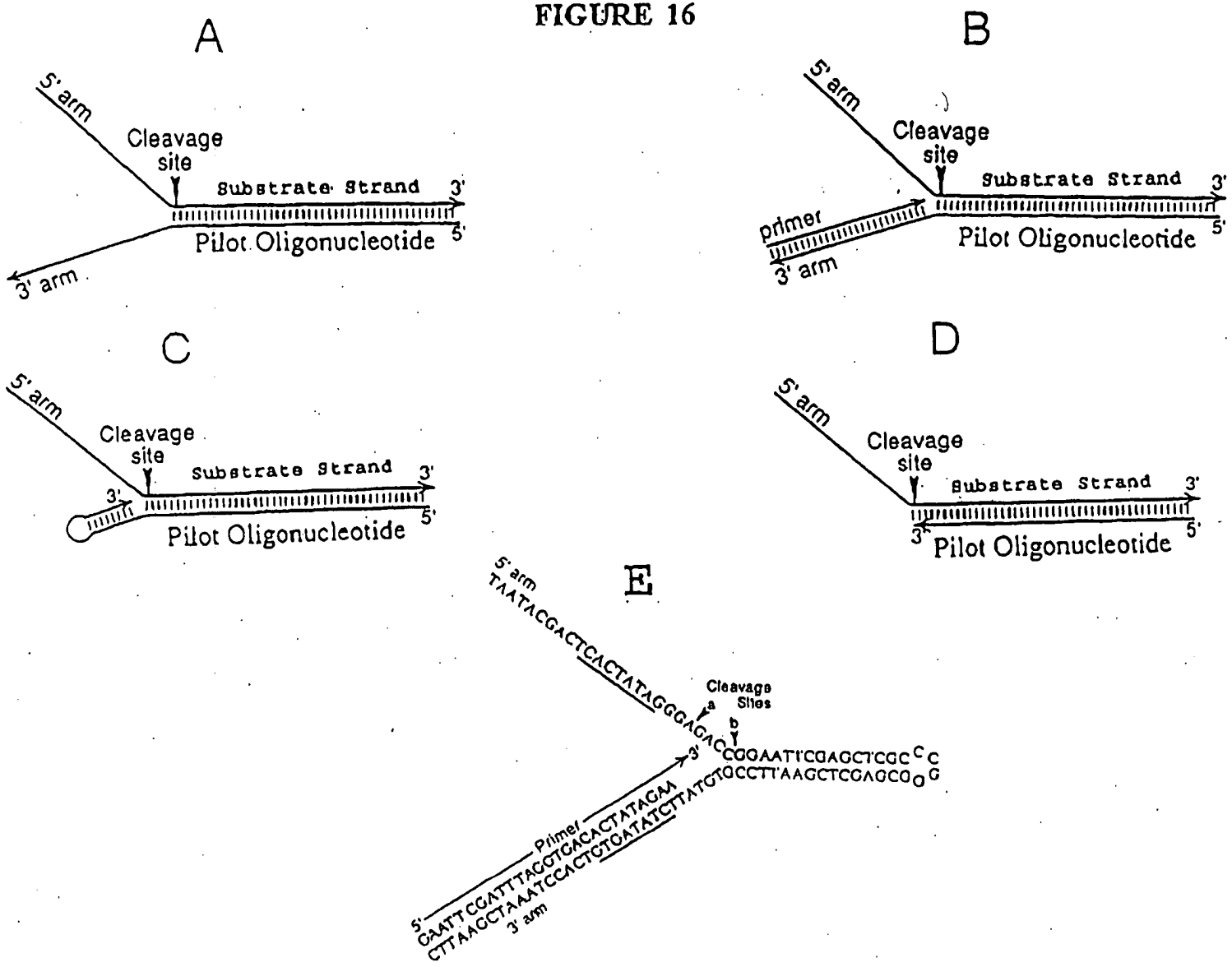

A
5' arm
Cleavage site
Substrate Strand
3'
Pilot Oligonucleotide
5'
3' arm
B
5' arm
Cleavage site
Substrate Strand
3'
primer
Pilot Oligonucleotide
5'
3' arm
C
5' arm
Cleavage site
Substrate Strand
3'
3'
Pilot Oligonucleotide
5'
D
5' arm
Cleavage site
Substrate Strand
3'
3'
Pilot Oligonucleotide
5'
E
5' arm
TAATACGACTCACTATAGGGAGACC
Cleavage Sites
a
b
3'
CGGAATTCGAGCTCGC C
GCCTTAAGCTCGAGCG G
Primer
5'
GAATTCGATTTAGGTGACACTATAGAA
CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTT
3' arm

FIGURE 17

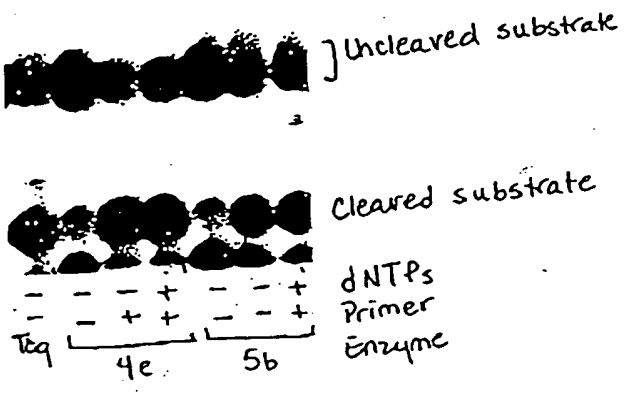

1 2 3 4 5 6 7
Uncleaved substrate
Cleared substrate
dNTPs
Primer
Enzyme
Taq
4e
5b

FIGURE 18

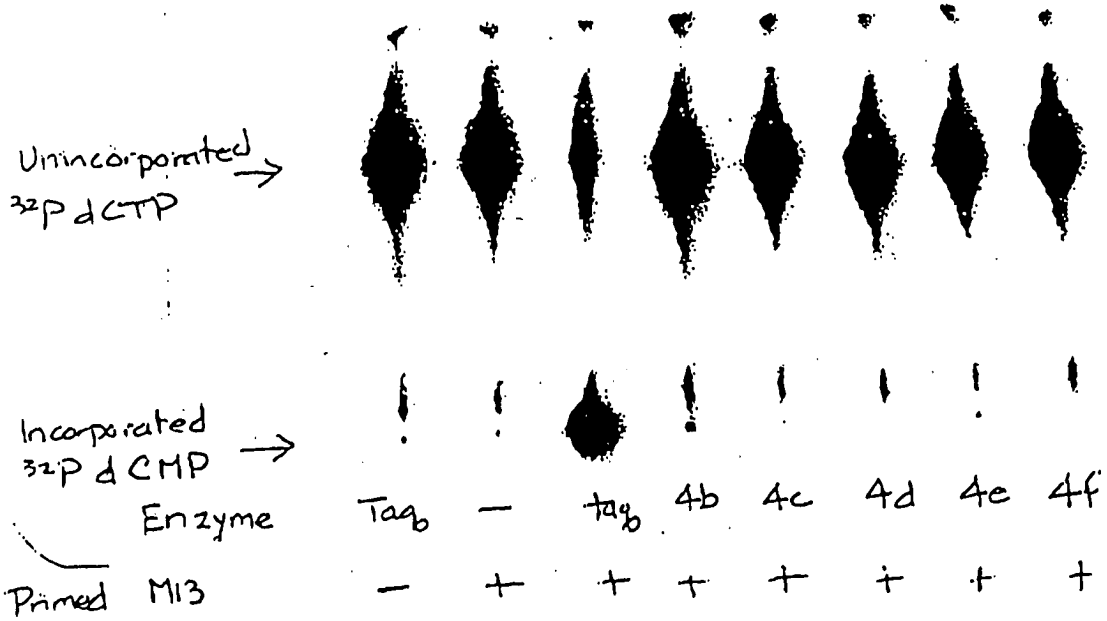
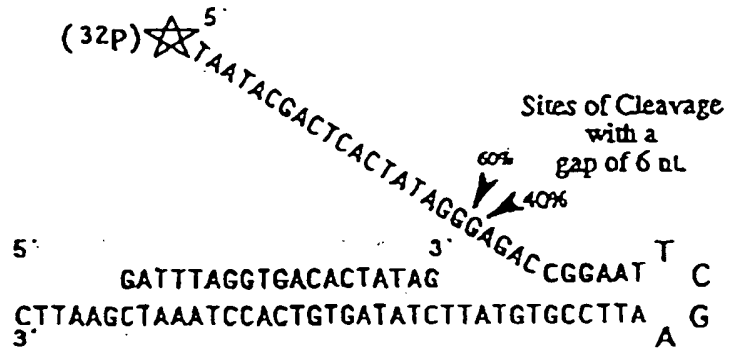

Unincorporated
32P dCTP
Incorporated
32P dCMP
Enzyme
Primed M13
Taq
—
tag
4b
4c
4d
4e
4f
—
+
+
+
+
+
+
+

FIGURE 19

A


(32P)
5'
TAATACGACTCACTATAGGGAGAC
Sites of Cleavage with a gap of 6 nt
60%
40%
5'
3'
GATTTAGGTGACACTATAG
CGGAAT
T
C
CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA
G
3'
A

B

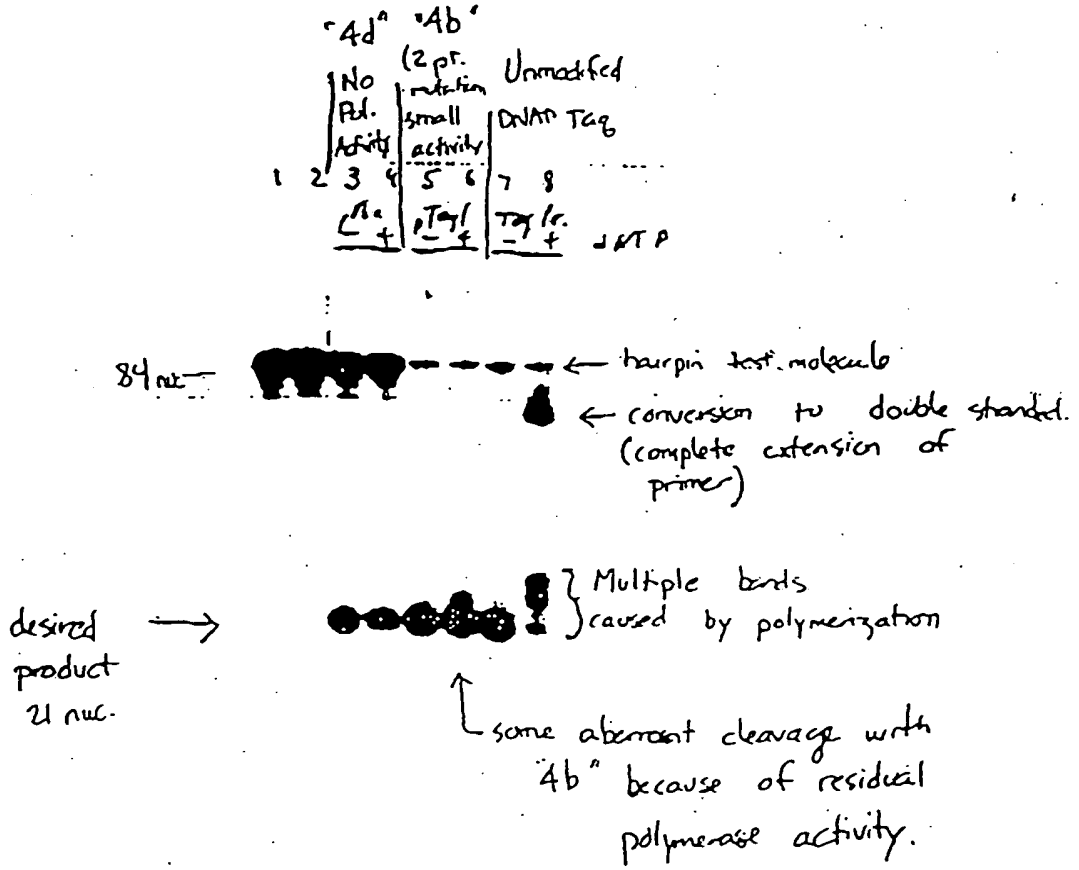
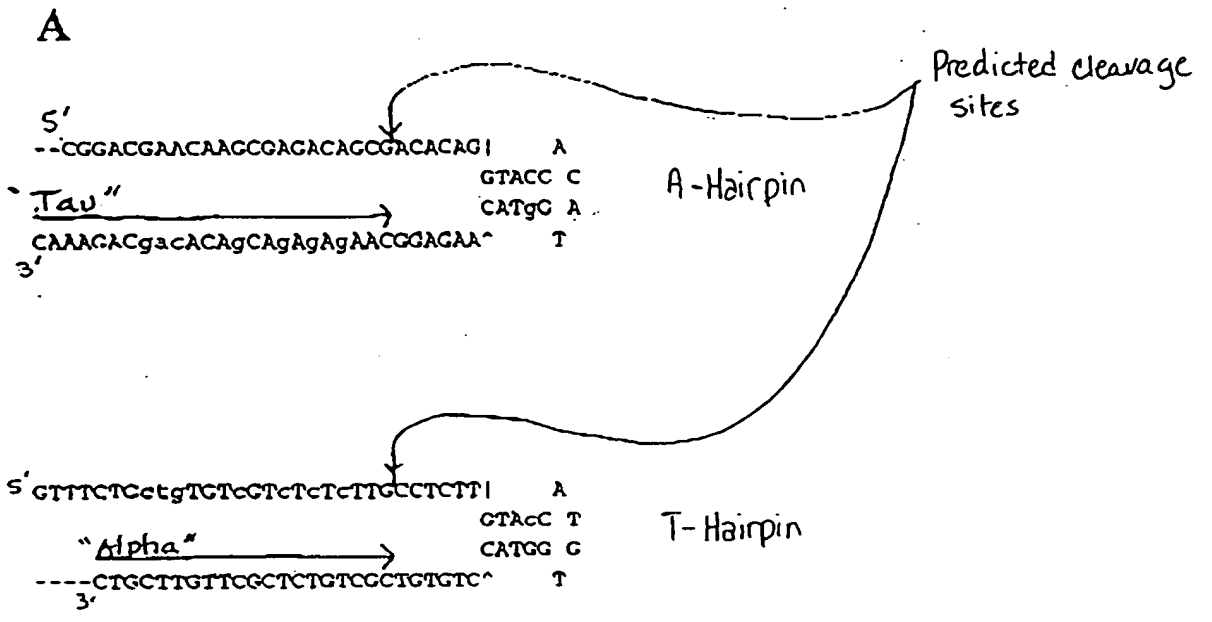

"4d"
"4b"
(2 pr. mutation small activity)
No Pol. Activity
Unmodified DNA Taq
1 2 3 4 5 6 7 8
Klenow + Taq I (-) Taq I (+) Taq Ir. (-) (+)
dNTP
84 nuc
hairpin test molecule
conversion to double stranded (complete extension of primer)
desired product 21 nuc.
Multiple bands caused by polymerization
some aberrant cleavage with "4b" because of residual polymerase activity.

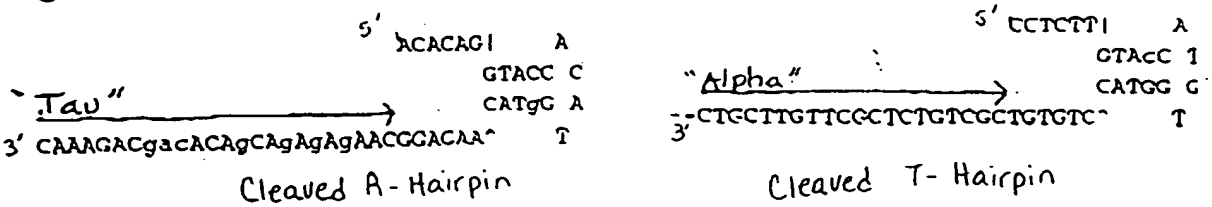
FIGURE 20



B Sequence of alpha primer:

5' GAC GAA CAA GCG AGA CAG CG 3'

C



D

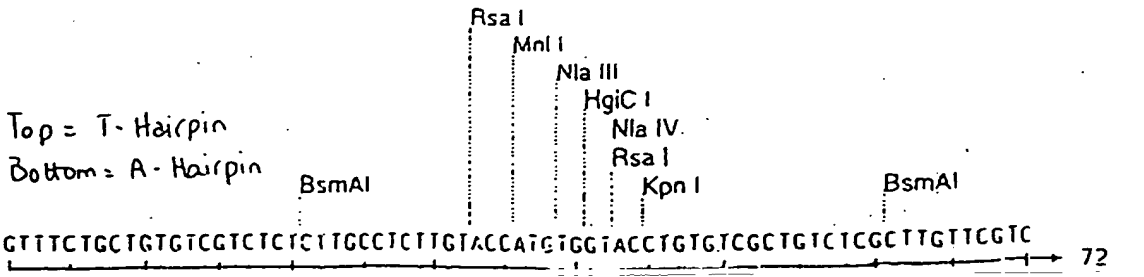


FIGURE 21

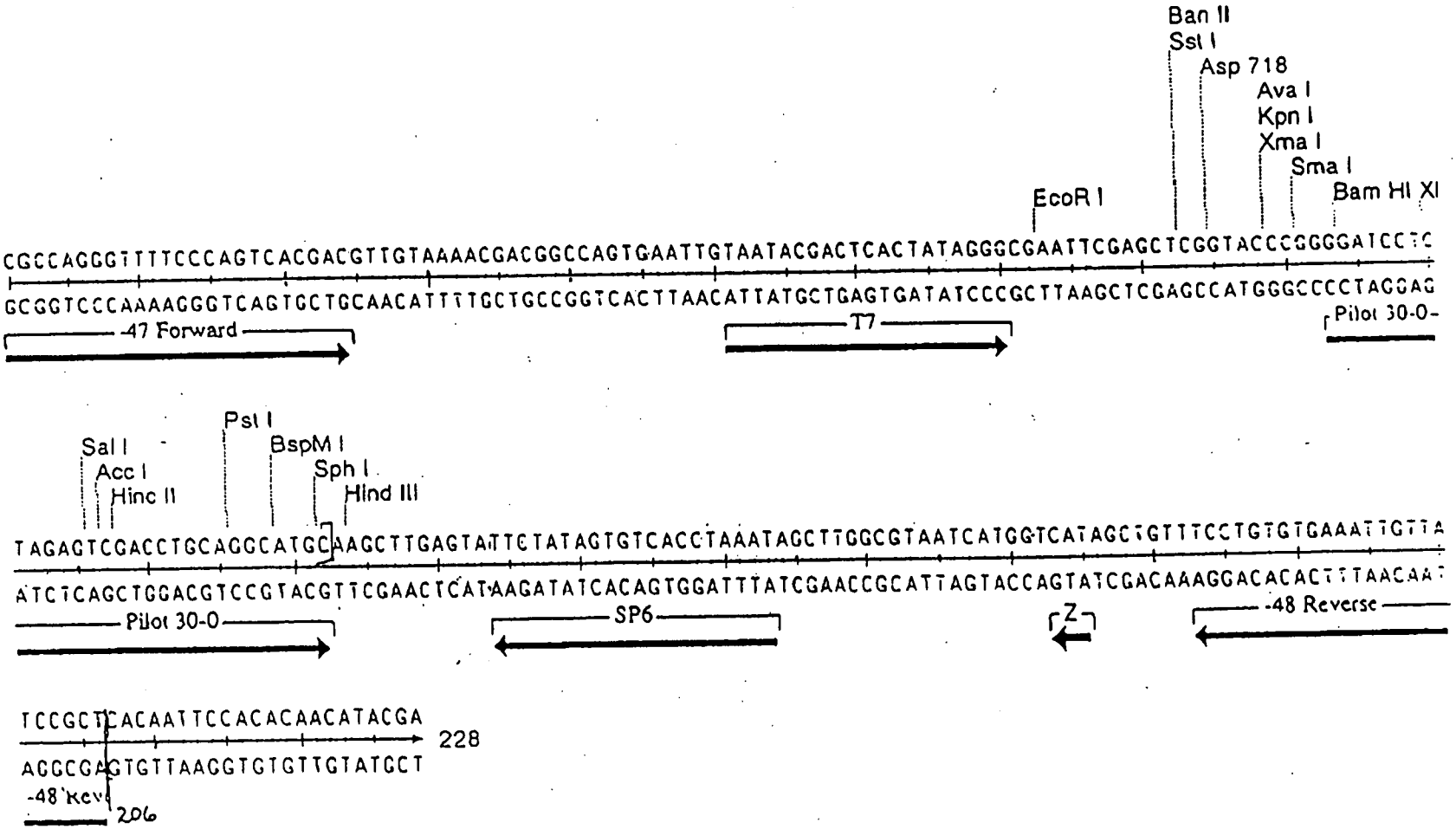

Ban II
Sst I
Asp 718
Ava I
Kpn I
Xma I
Sma I
Bam HI XI
EcoR I
CGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTCGAGCTCGGTACCCGGGGATCCTC
GCGGTCCCAAAAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCACTTAACATTATGCTGAGTGATATCCCGCTTAAGCTCGAGCCATGGGCCCCTAGGAG
-47 Forward
T7
Pilot 30-0-
Pst I
Sal I
BspM I
Acc I
Sph I
Hinc II
Hind III
TAGAGTCGACCTGCAGGCATGCAAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTA
ATCTCAGCTGGACGTCCGTACGTTCGAACTCATAAGATATCACAGTGGATTTATCGAACCGCATTAGTACCAGTATCGACAAAGGACACACTTTAACAAT
Pilot 30-0
SP6
Z
-48 Reverse
TCCGCTCACAATTCCACACAACATACGA
228
AGGCGAGTGTTAAGGTGTGTTGTATGCT
-48 Rev
206

FIGURE 22A

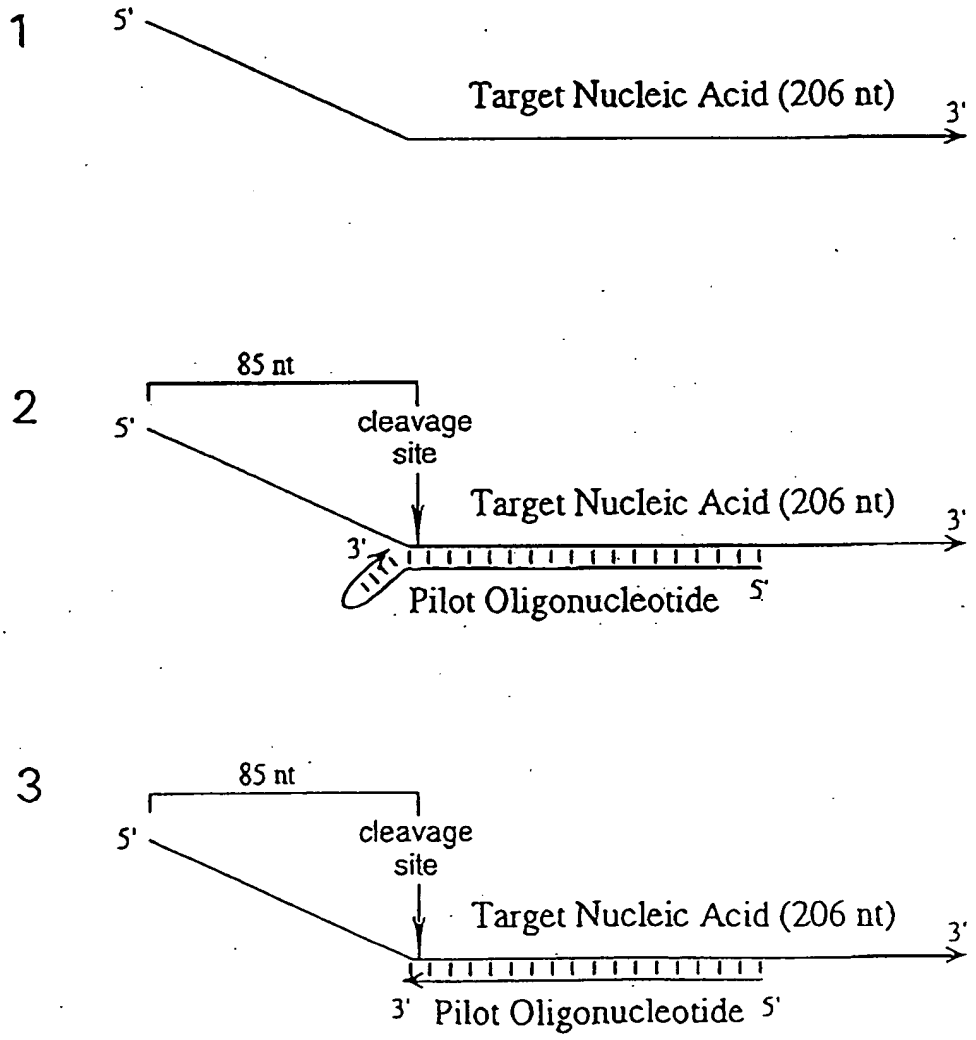

1
5'
Target Nucleic Acid (206 nt)
3'
2
85 nt
5'
cleavage site
Target Nucleic Acid (206 nt)
3'
3'
Pilot Oligonucleotide
5'
3
85 nt
5'
cleavage site
Target Nucleic Acid (206 nt)
3'
3'
Pilot Oligonucleotide
5'

FIGURE 22B

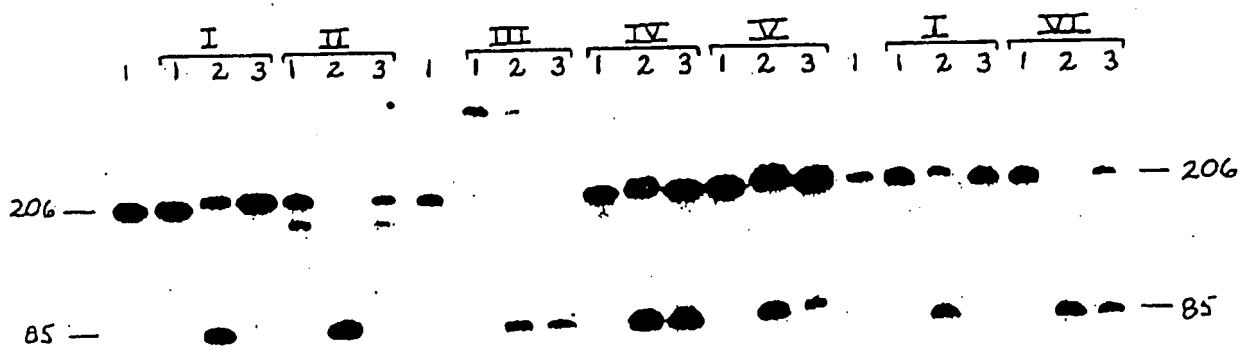


FIGURE 23

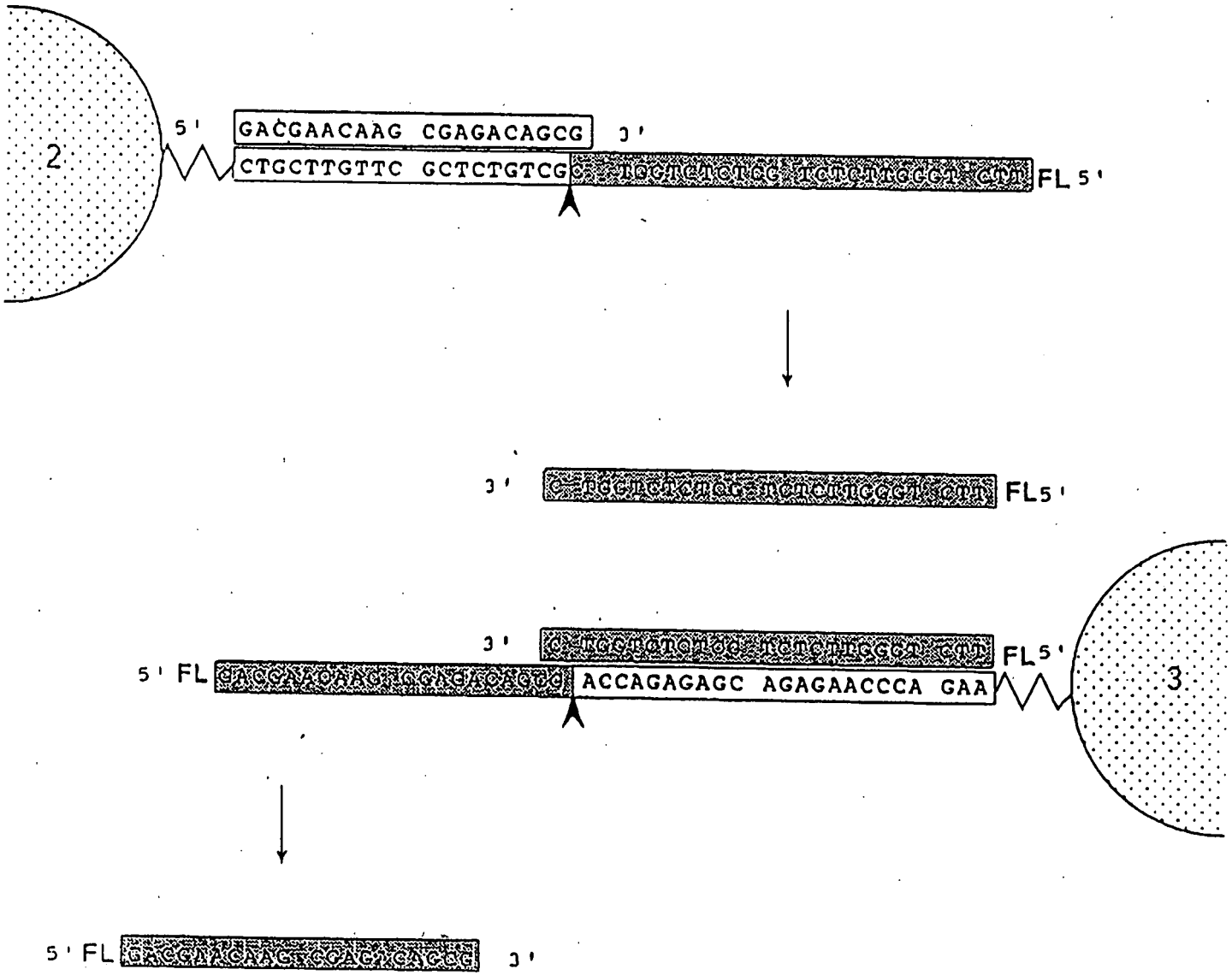

2
5'
GACGAACAAG CGAGACAGCG
3'
CTGCTTGTTC GCTCTGTCGC
FL 5'
3'
FL5'
5' FL
ACCAGAGAGC AGAGAACCCA GAA
FL 5'
3
5' FL
3'

FIGURE 24

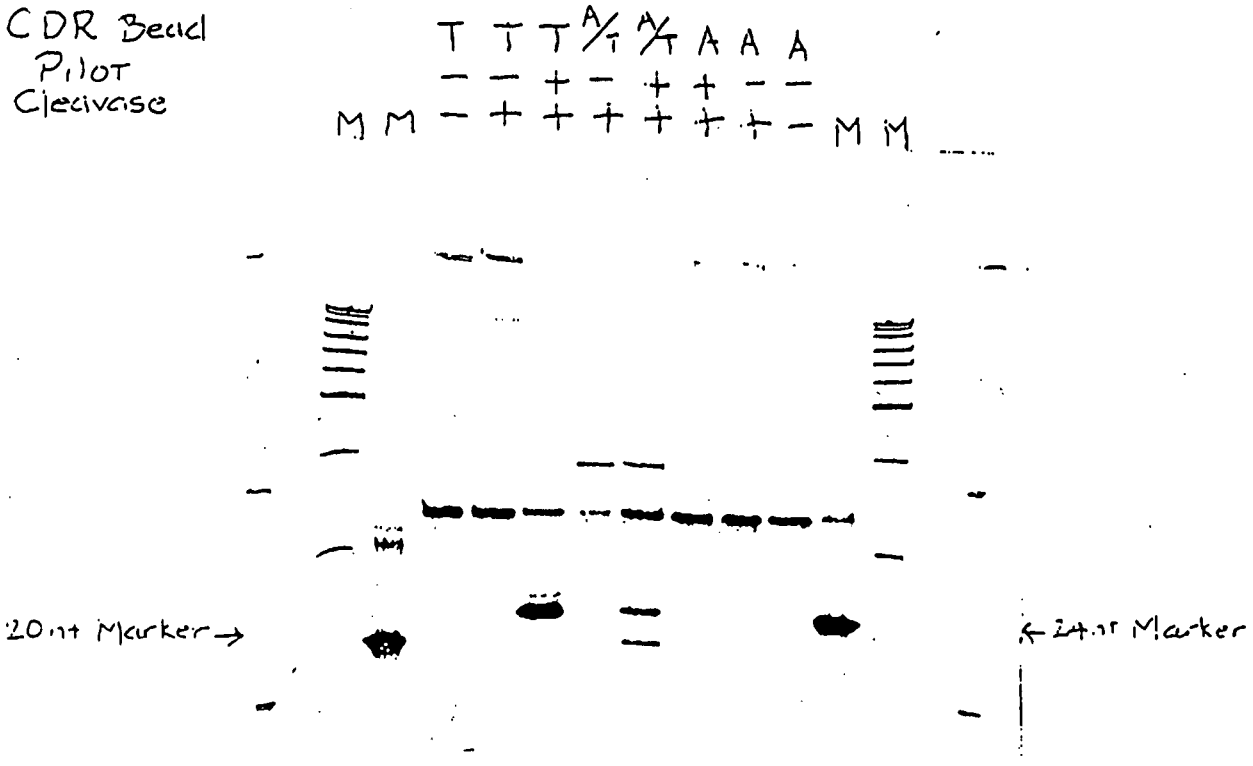

CDR Beacl
Pilot
Cleavase
T T T A/T A/T A A A
M M
M M
20 nt Marker →
← 24 nt Marker

FIGURE 25

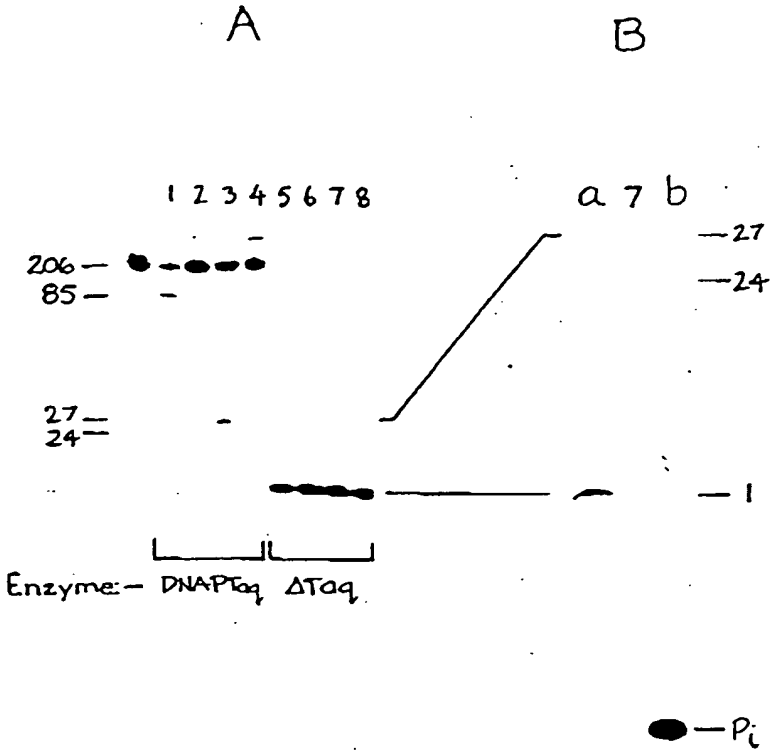
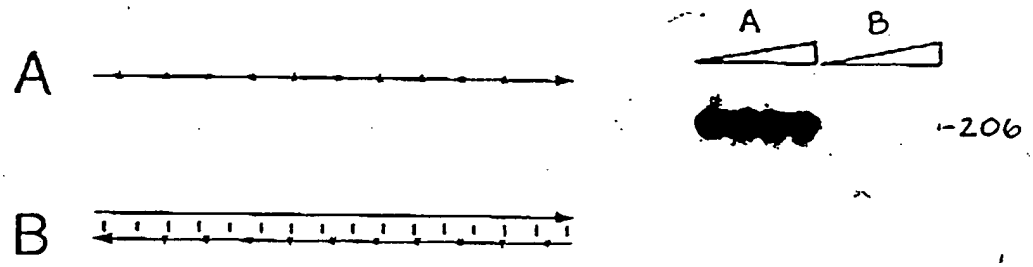


FIGURE 26



• = ^{32}P

FIGURE 27

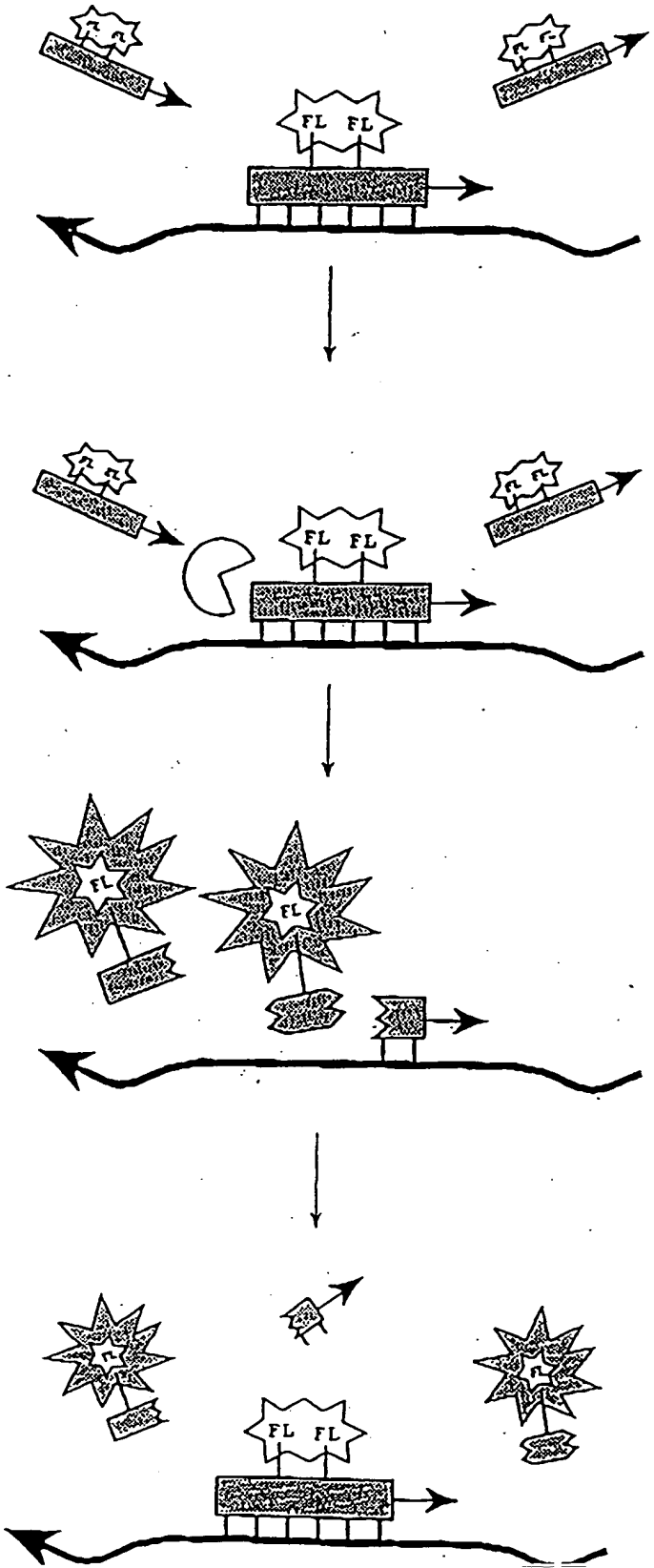
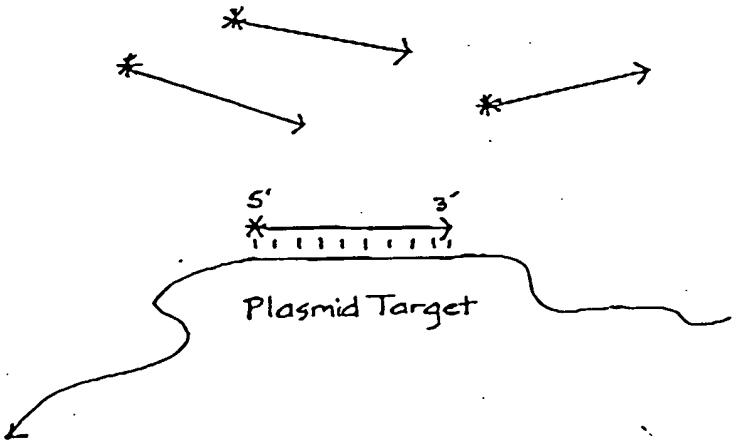


FIGURE 28A



* = ^{32}P 5' terminal phosphate

FIGURE 28B

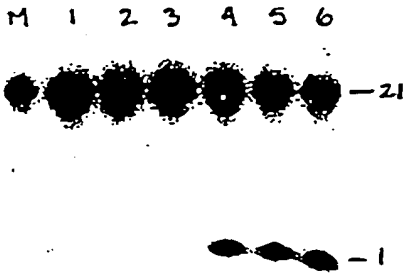


FIGURE 29

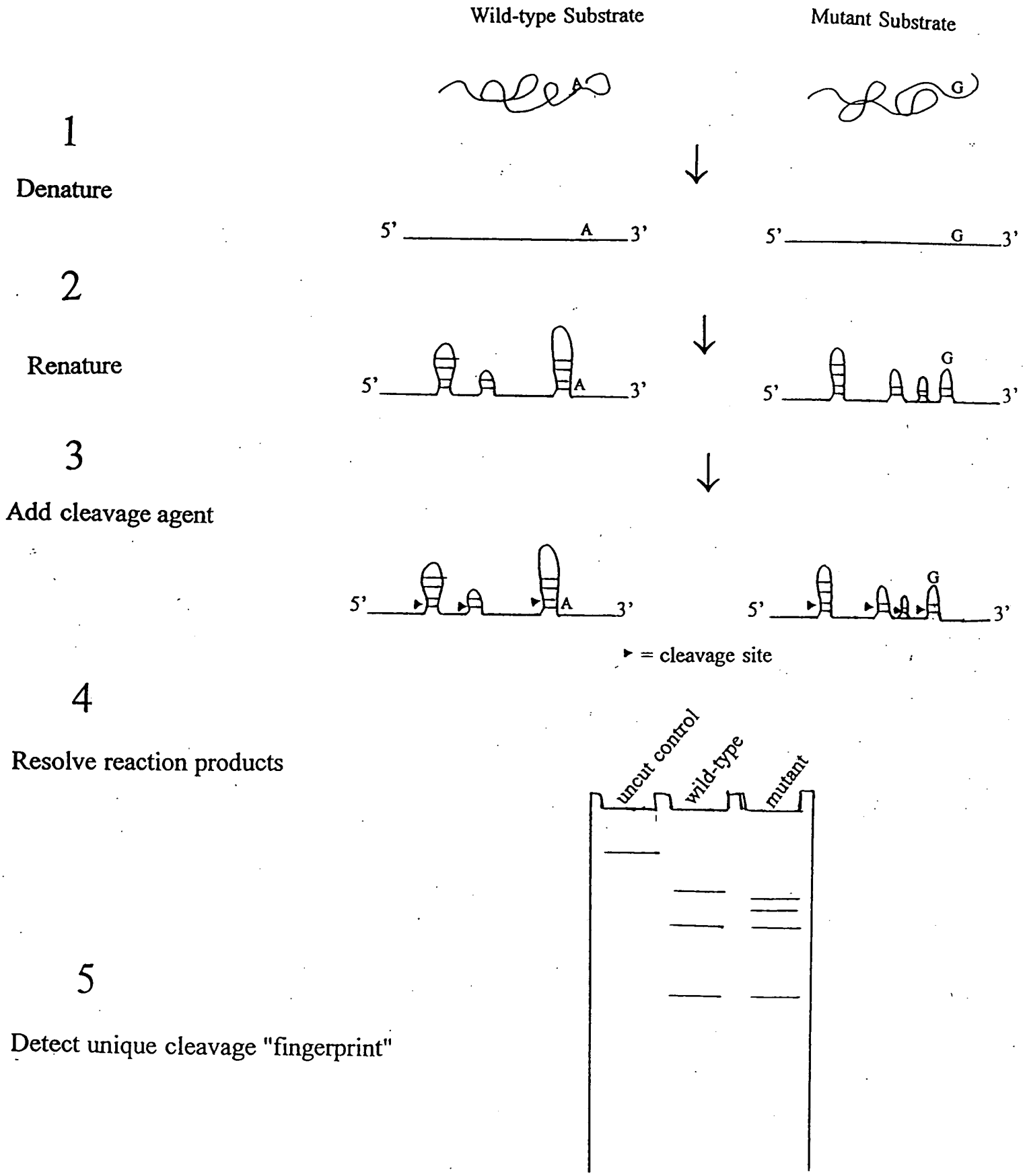

Wild-type Substrate
Mutant Substrate
1
Denature
A
G
5'
3'
2
Renature
3
Add cleavage agent
▸ = cleavage site
4
Resolve reaction products
uncut control
wild-type
mutant
5
Detect unique cleavage "fingerprint"

FIGURE 30

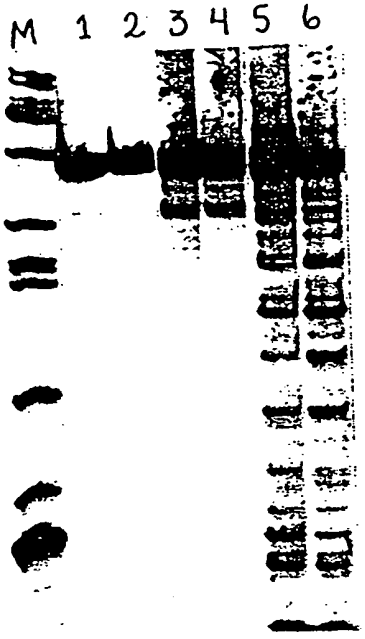


FIGURE 31

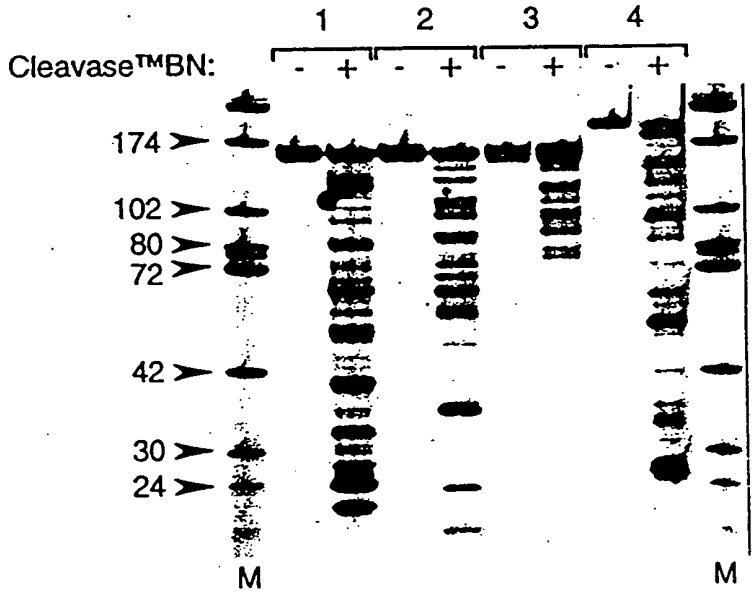


FIGURE 32

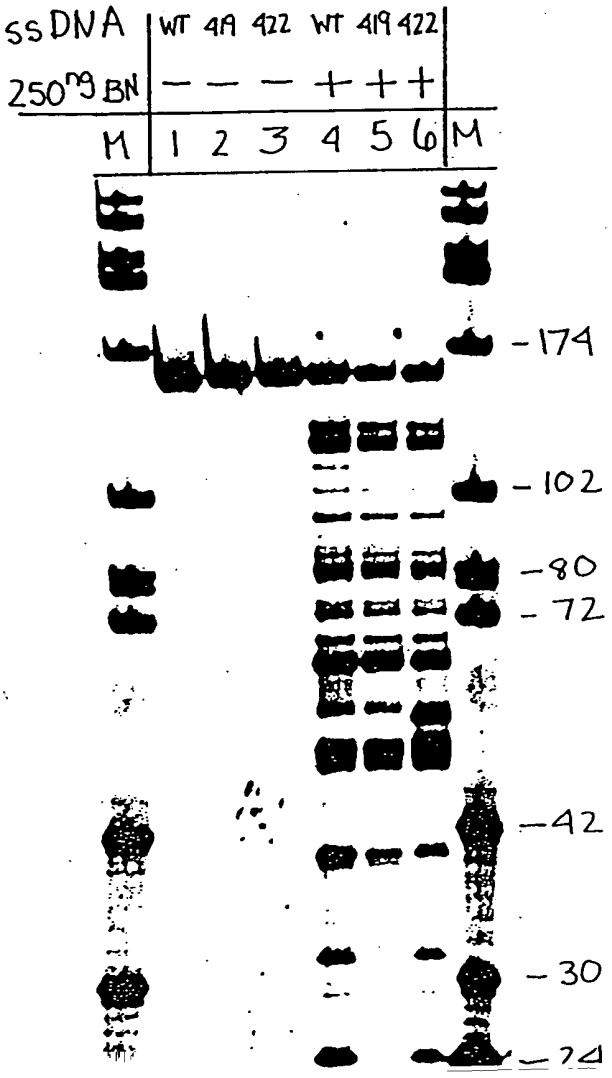

ss DNA
WT 4A 422 WT 4A9 422
250 ng BN
— — — + + +
M 1 2 3 4 5 6 M
-174
-102
-80
-72
-42
-30
-24

FIGURE 33

FIGURE 34

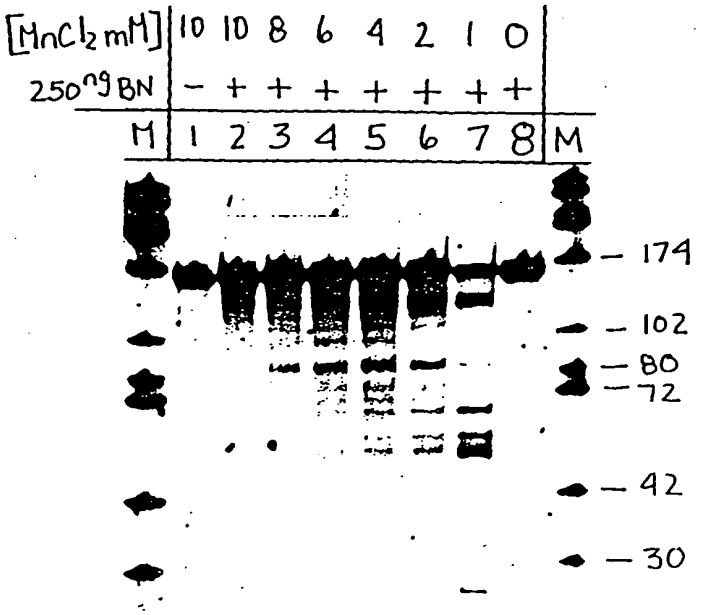


FIGURE 35

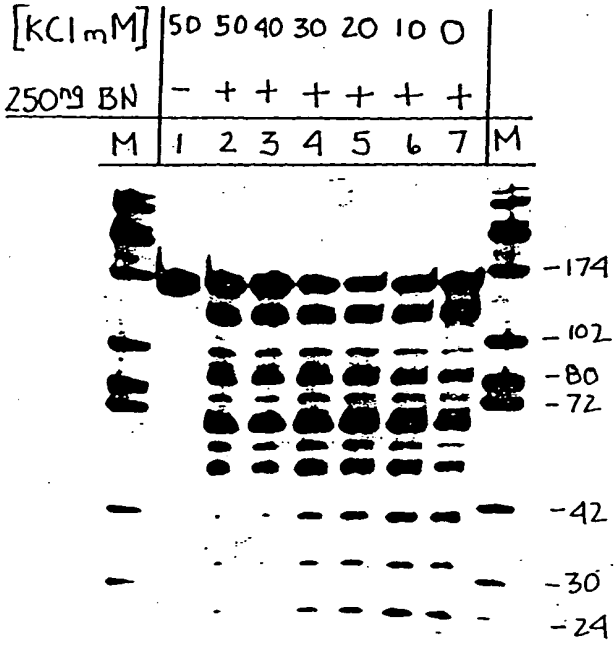

[KCl mM]
50 50 40 30 20 10 0
250ng BN
- + + + + + +
M
1 2 3 4 5 6 7
M
174
102
80
72
42
30
24

FIGURE 36

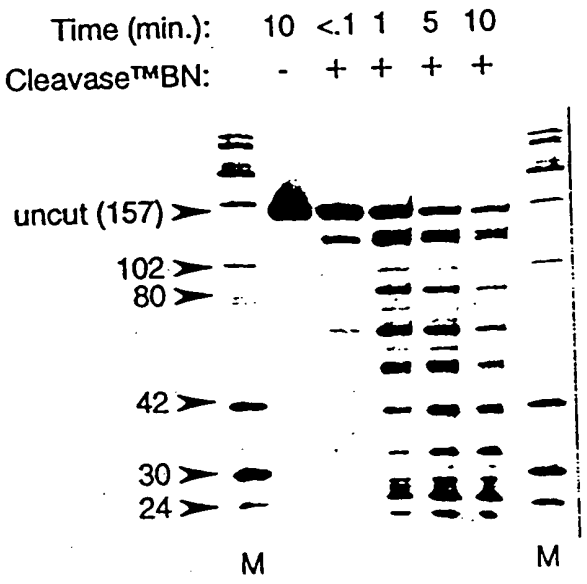


FIGURE 37

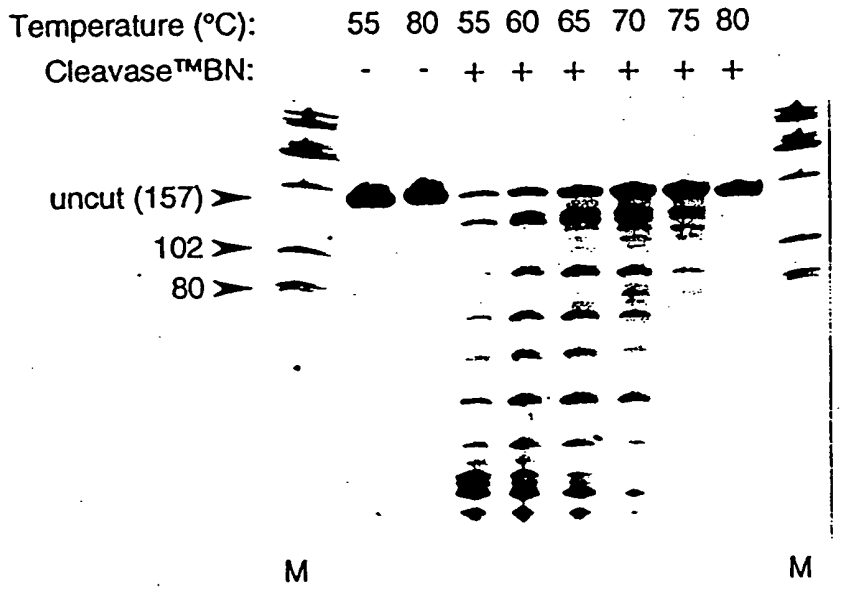

Temperature (°C): 55 80 55 60 65 70 75 80
Cleavase™BN: - - + + + + + +
uncut (157)
102
80
M
M

FIGURE 38

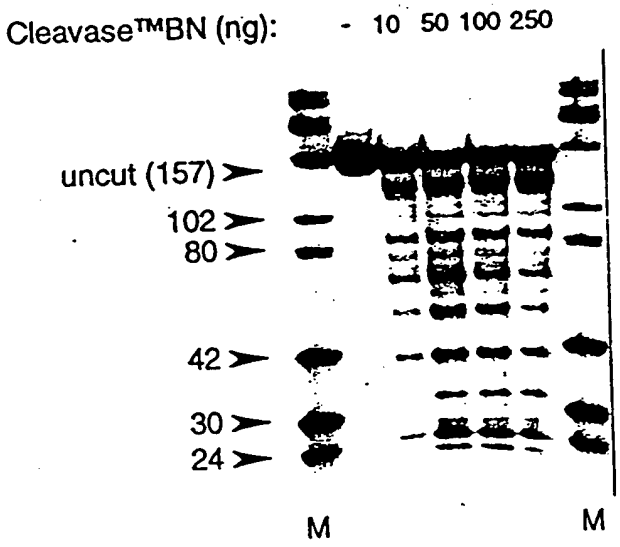


FIGURE 39

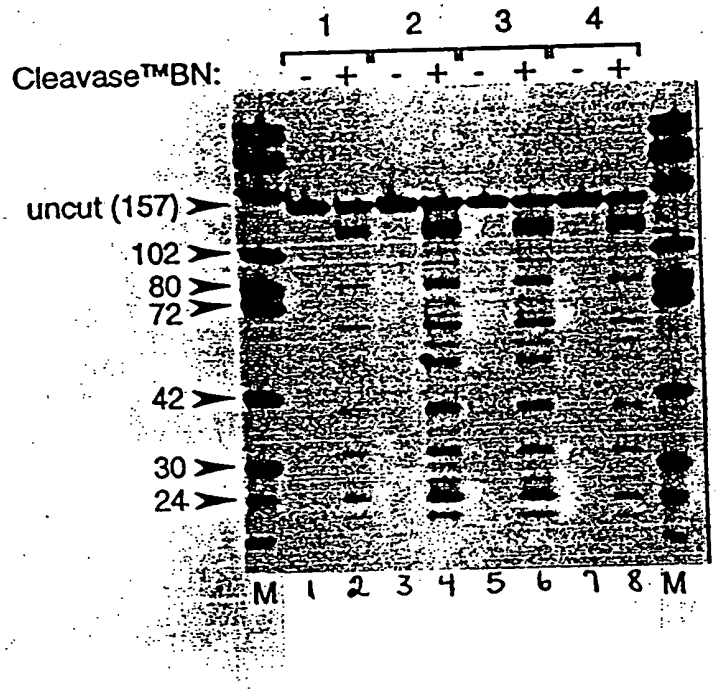


FIGURE 40

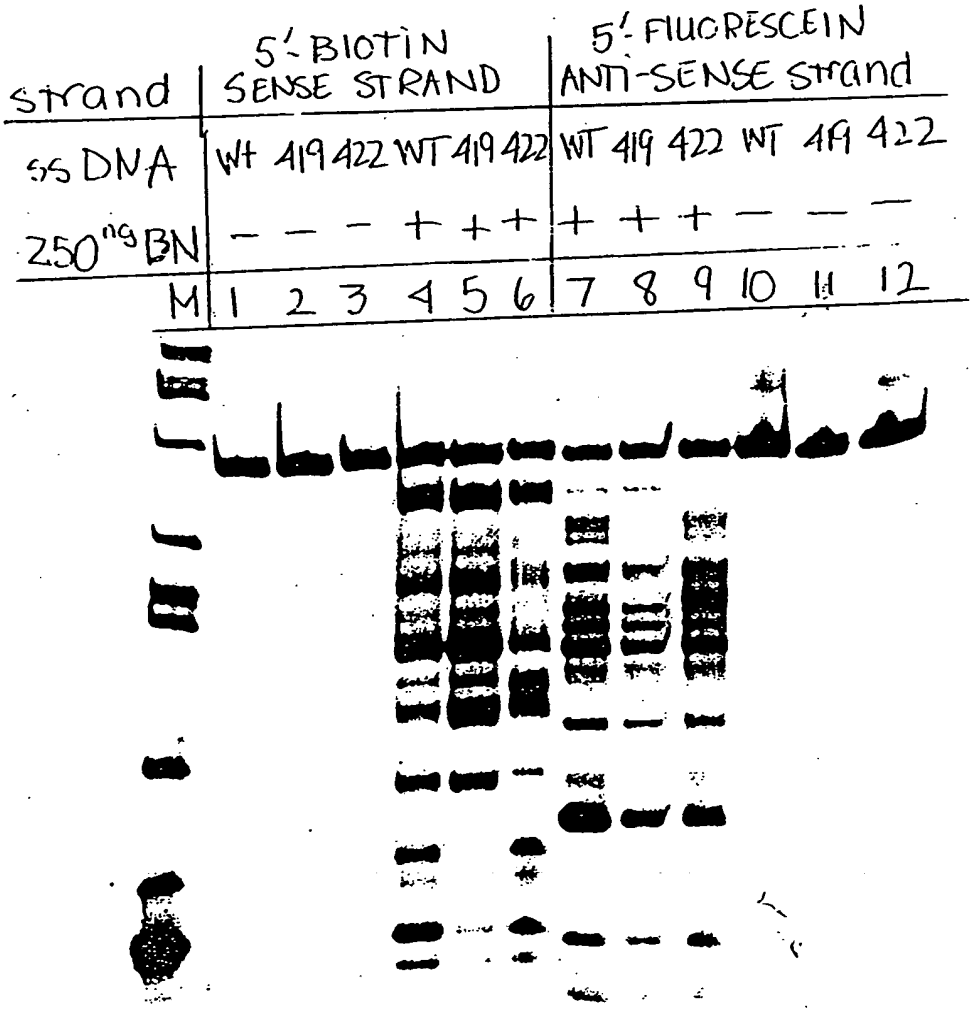

5'-BIOTIN
SENSE STRAND
5'-FLUORESCEIN
ANTI-SENSE STRAND
strand
ss DNA
250 ng BN
WT 419 422 WT 419 422
WT 419 422 WT 419 422
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 41

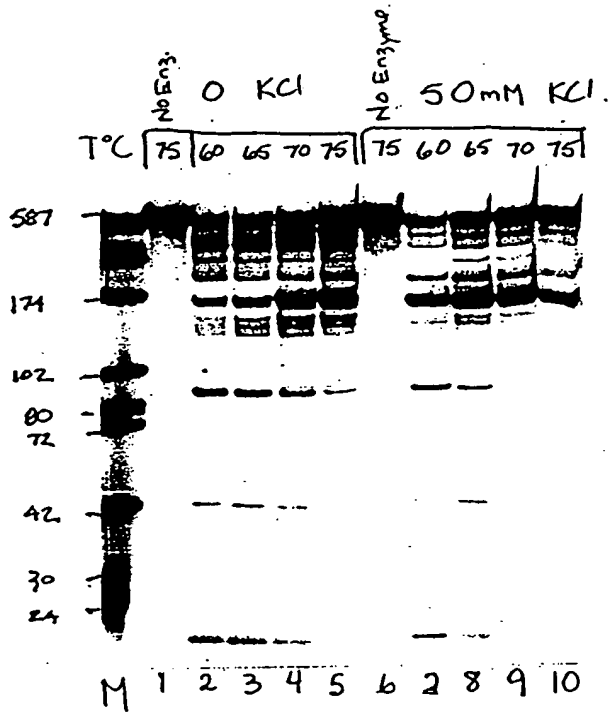

No Enz.
0 KCl
No Enzyme
50mM KCl.
T°C
75
60
65
70
75
75
60
65
70
75
587
174
102
80
72
42
30
24
M
1
2
3
4
5
6
2
8
9
10

FIGURE 42

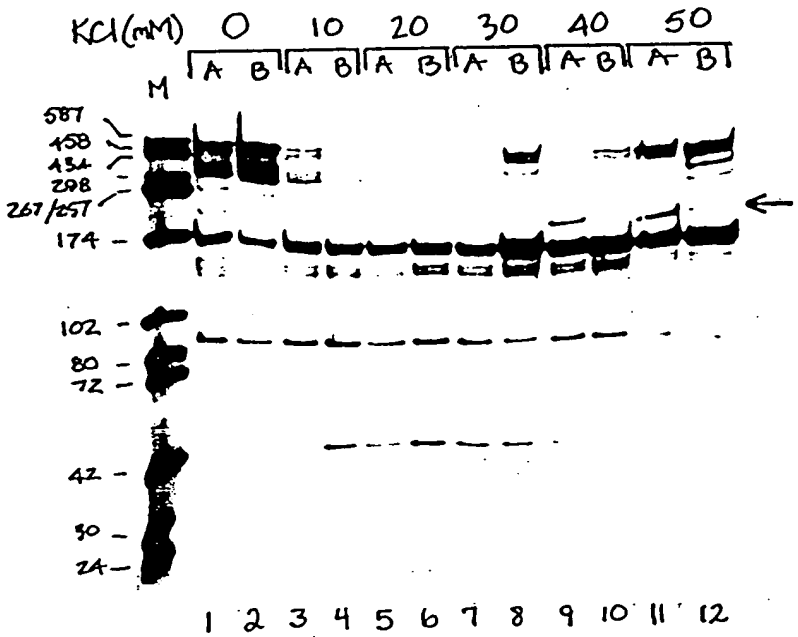

KCl(mM) 0 10 20 30 40 50
A B A B A B A B A B A B
M
587
458
434
298
267/257
174
102
80
72
42
30
24
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 43

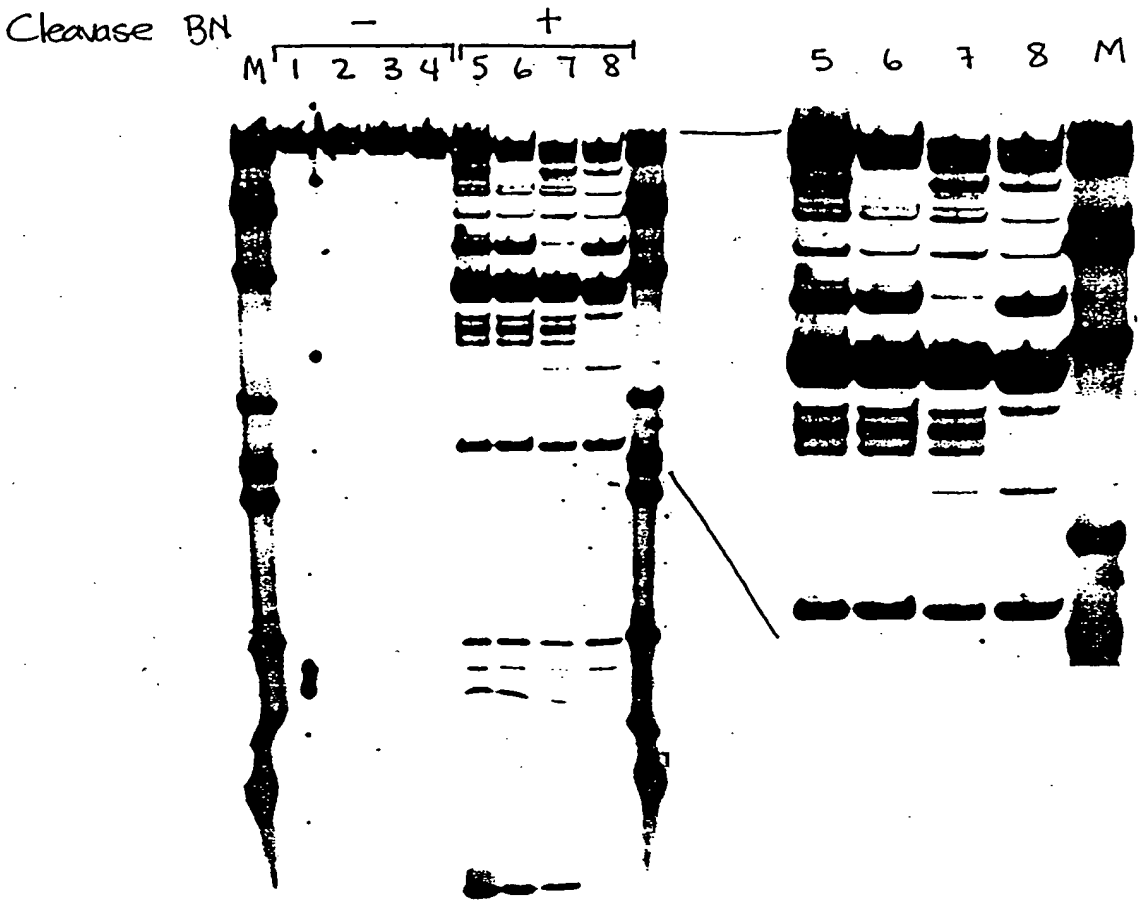


FIGURE 44

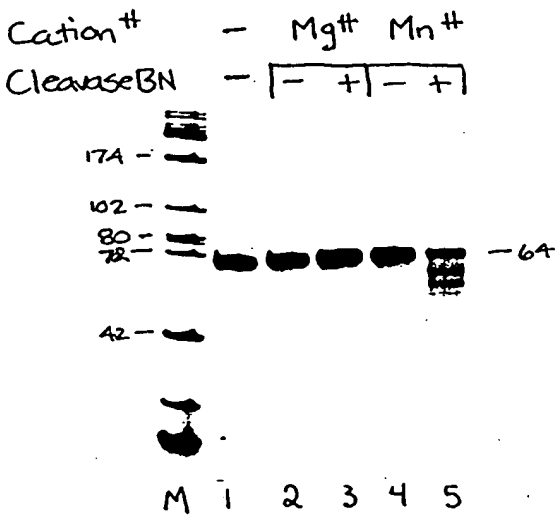


FIGURE 45

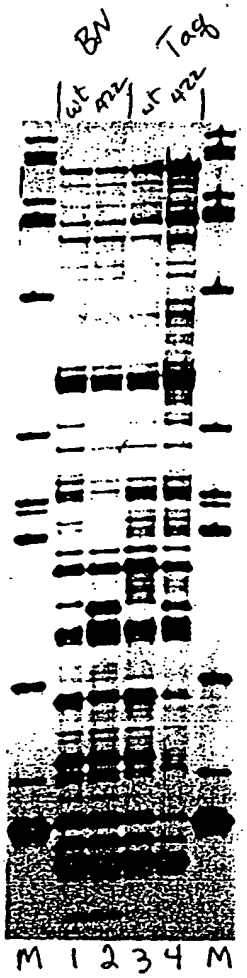


FIGURE 46

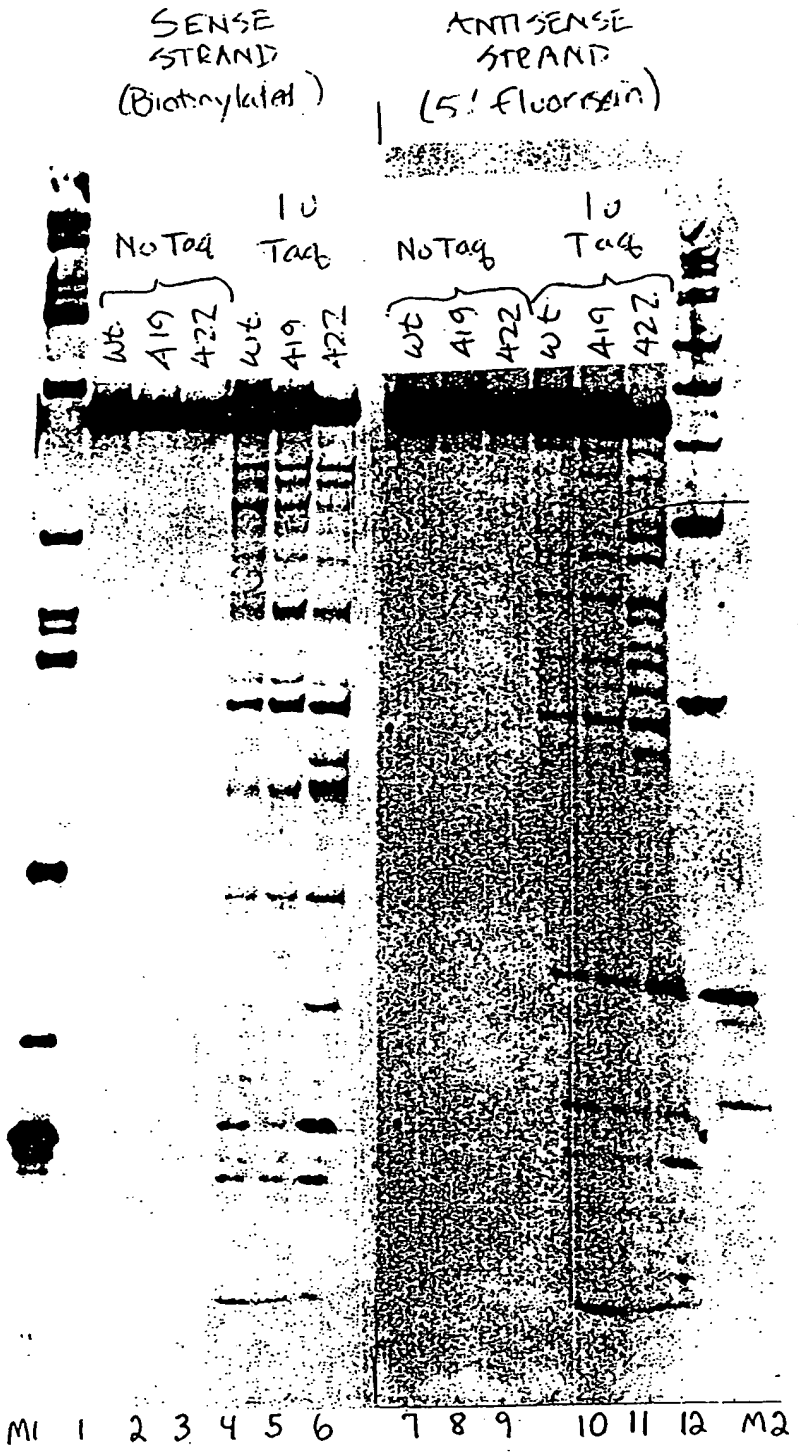

SENSE STRAND (Biotinylated)
ANTISENSE STRAND (5' fluorescein)
No Taq
Taq
wt
419
422
M1
1
2
3
4
5
6
7
8
9
10
11
12
M2

FIGURE 47

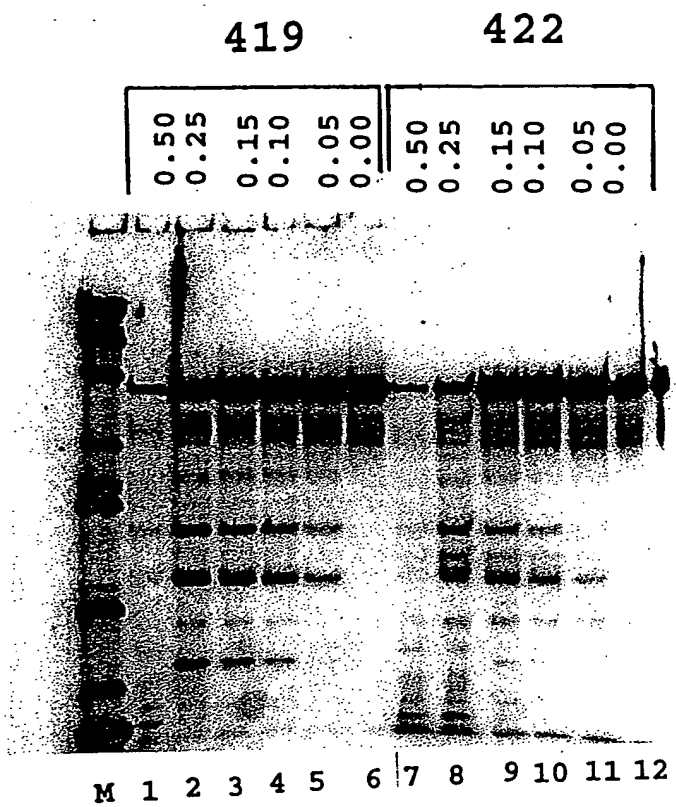

419
422
0.50
0.25
0.15
0.10
0.05
0.00
0.50
0.25
0.15
0.10
0.05
0.00
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 48

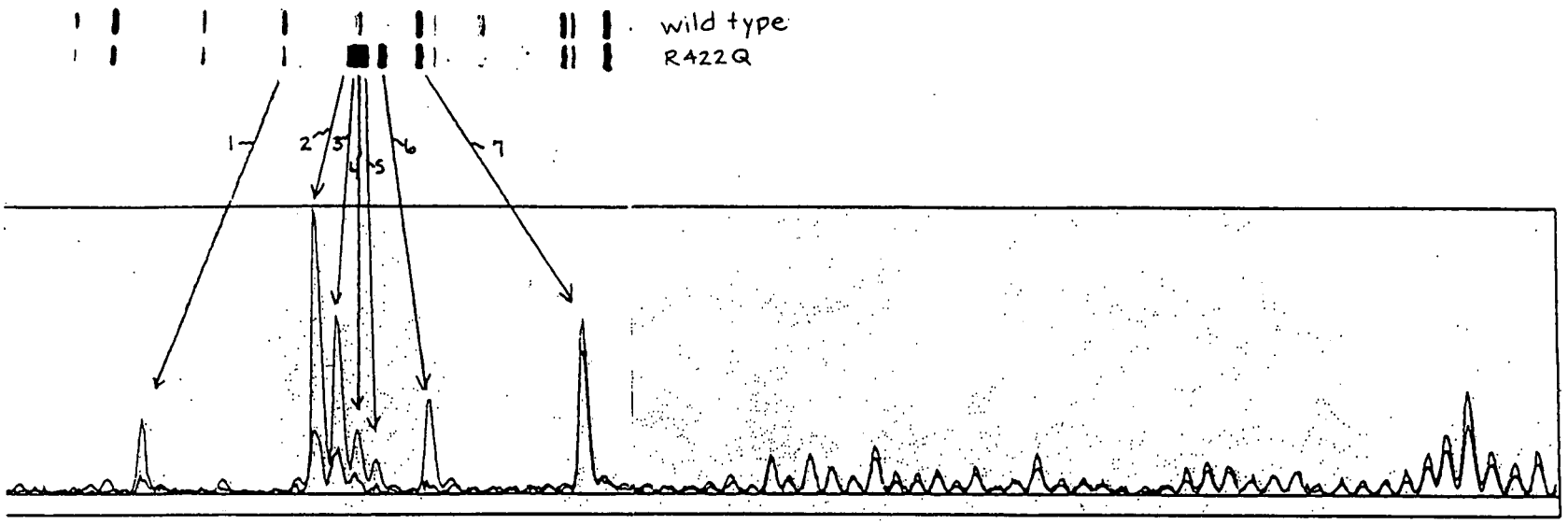


FIGURE 49

```
                                                  50                                                  100
L.100.8-1   5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG ATGTTACGGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO:76)  3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC TACAATGCCCCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGTGAGAGA

L.46.16-10  5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGACTTTCCACAAGGGG ATGTTATGGGGAGG----------AGCCGGTCGGGAACACCCACTTTCT
(SEQ ID NO:77)  3'CCGACTGTTCTTCCTTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC TACAATACCCCTCC----------TCGGCCAGCCCTTGTGGGTGAAAGA

L.46.16-12  5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGACTTTCCACAAGGGG ATGTTATGGGGAGG----------AGCCGGTCGGGAACACCCACTTTCT
(SEQ ID NO:78)  3'CCGACTGTTCTTCCTTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC TACAATACCCCTCC----------TCGGCCAGCCCTTGTGGGTGAAAGA

L.19.16-3   5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG ATGTTACGGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCCCTCTCT
(SEQ ID NO:79)  3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC TACAATGCCCCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGGGAGAGA

L.CEM/251   5'GGCTGACAAGAAGGAAACTCGCTGAAACAGCAGGGACTTTCCACAAGGGG ATGTTACGGGGAGGTACTGGGAAGGAGCCGGTCGGGAACGCCCACTTTCT
(SEQ ID NO:80)  3'CCGACTGTTCTTCCTTTGAGCGACTTTGTCGTCCCTGAAAGGTGTTCCCC TACAATGCCCCTCCATGACCCTTCCTCGGCCAGCCCTTGCGGGTGAAAGA

L.36.8-3    5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG ATGTTACGGAGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO:81)  3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC TACAATGCCTCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGTGAGAGA


                                                  150                                                 200
L.100.8-1   5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
            3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.46.16-10  5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
            3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.46.16-12  5'TGGTGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
            3'ACCACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.19.16-3   5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
            3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.CEM/251   5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
            3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.36.8-3    5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA GAGGCTGGCAGATTGAGCCCTAGGAGGTTCTCTCCAGCACTAGCAGGTAG
            3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT CTCCGACCGTCTAACTCGGGATCCTCCAAGAGAGGTCGTGATCGTCCATC
```

FIGURE 49

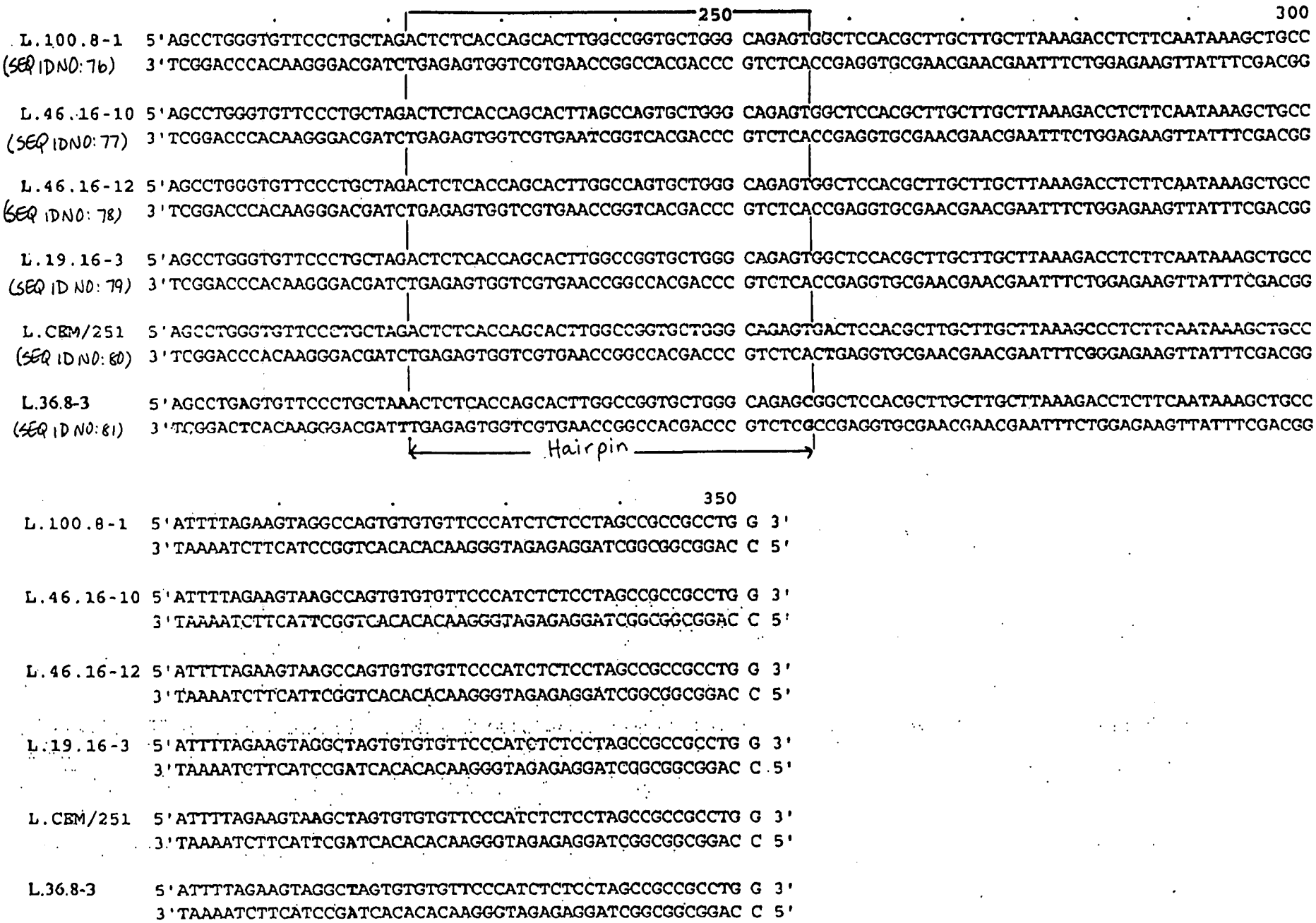

250
300
L.100.8-1 5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCGGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
(SEQ ID NO: 76) 3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCACCGAGGTGCGAACGAACGAATTTCTGGAGAAGTTATTTCGACGG
L.46.16-10 5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTAGCCAGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
(SEQ ID NO: 77) 3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAATCGGTCACGACCC GTCTCACCGAGGTGCGAACGAACGAATTTCTGGAGAAGTTATTTCGACGG
L.46.16-12 5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCAGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
(SEQ ID NO: 78) 3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGTCACGACCC GTCTCACCGAGGTGCGAACGAACGAATTTCTGGAGAAGTTATTTCGACGG
L.19.16-3 5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCGGTGCTGGG CAGAGTGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
(SEQ ID NO: 79) 3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCACCGAGGTGCGAACGAACGAATTTCTGGAGAAGTTATTTCGACGG
L.CEM/251 5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCGGTGCTGGG CAGAGTGACTCCACGCTTGCTTGCTTAAAGCCCTCTTCAATAAAGCTGCC
(SEQ ID NO: 80) 3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCACTGAGGTGCGAACGAACGAATTTCGGGAGAAGTTATTTCGACGG
L.36.8-3 5'AGCCTGAGTGTTCCCTGCTAAACTCTCACCAGCACTTGGCCGGTGCTGGG CAGAGCGGCTCCACGCTTGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
(SEQ ID NO: 81) 3'TCGGACTCACAAGGGACGATTTGAGAGTGGTCGTGAACCGGCCACGACCC GTCTCGCCGAGGTGCGAACGAACGAATTTCTGGAGAAGTTATTTCGACGG
Hairpin
350
L.100.8-1 5'ATTTTAGAAGTAGGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATCCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.46.16-10 5'ATTTTAGAAGTAAGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.46.16-12 5'ATTTTAGAAGTAAGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.19.16-3 5'ATTTTAGAAGTAGGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATCCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.CEM/251 5'ATTTTAGAAGTAAGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATTCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.36.8-3 5'ATTTTAGAAGTAGGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATCCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

FIGURE 50

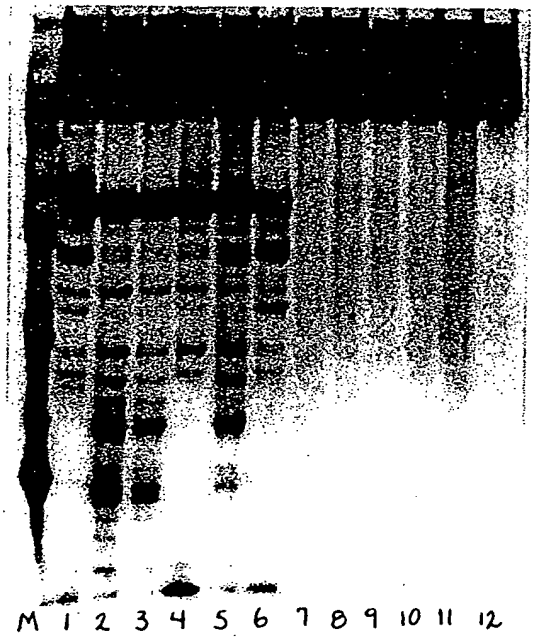


FIGURE 51

FIGURE 52

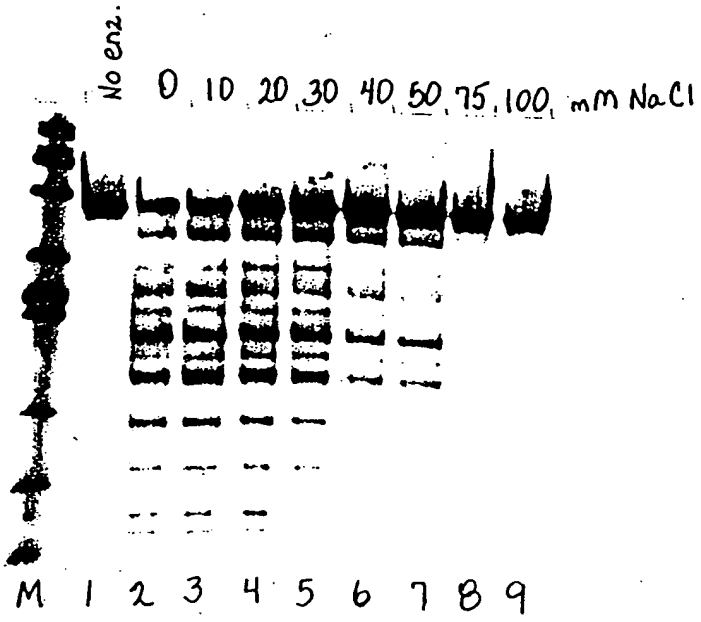


FIGURE 53

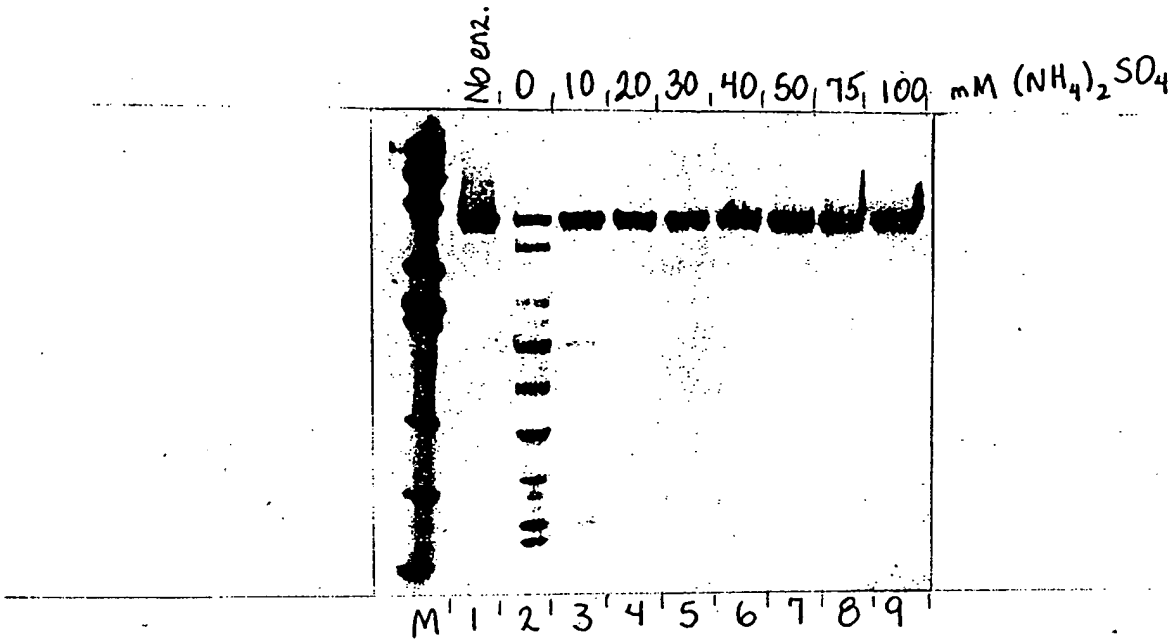


FIGURE 54

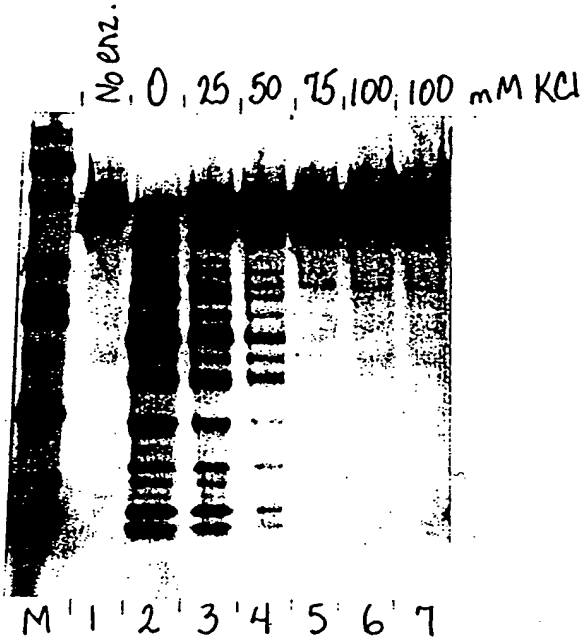


FIGURE 55

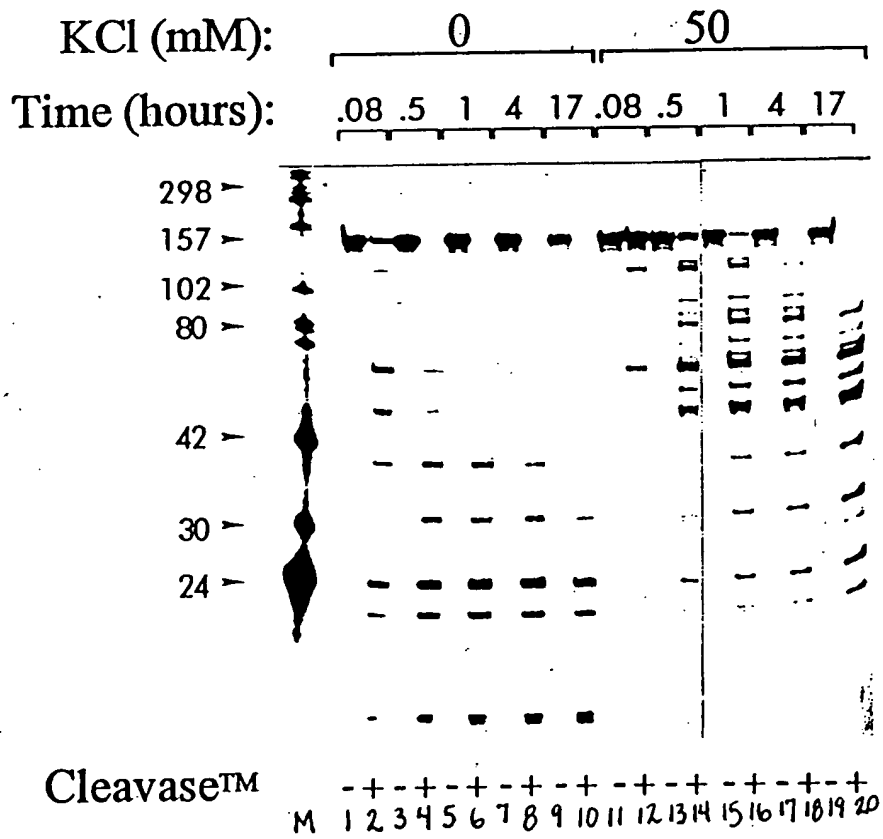

KCl (mM):
0
50
Time (hours):
.08 .5 1 4 17 .08 .5 1 4 17
298
157
102
80
42
30
24
Cleavase™
- + - + - + - + - + - + - + - + - + - +
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

FIGURE 56

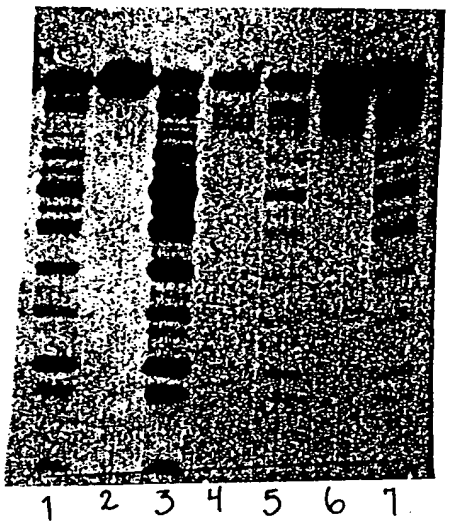


FIGURE 57

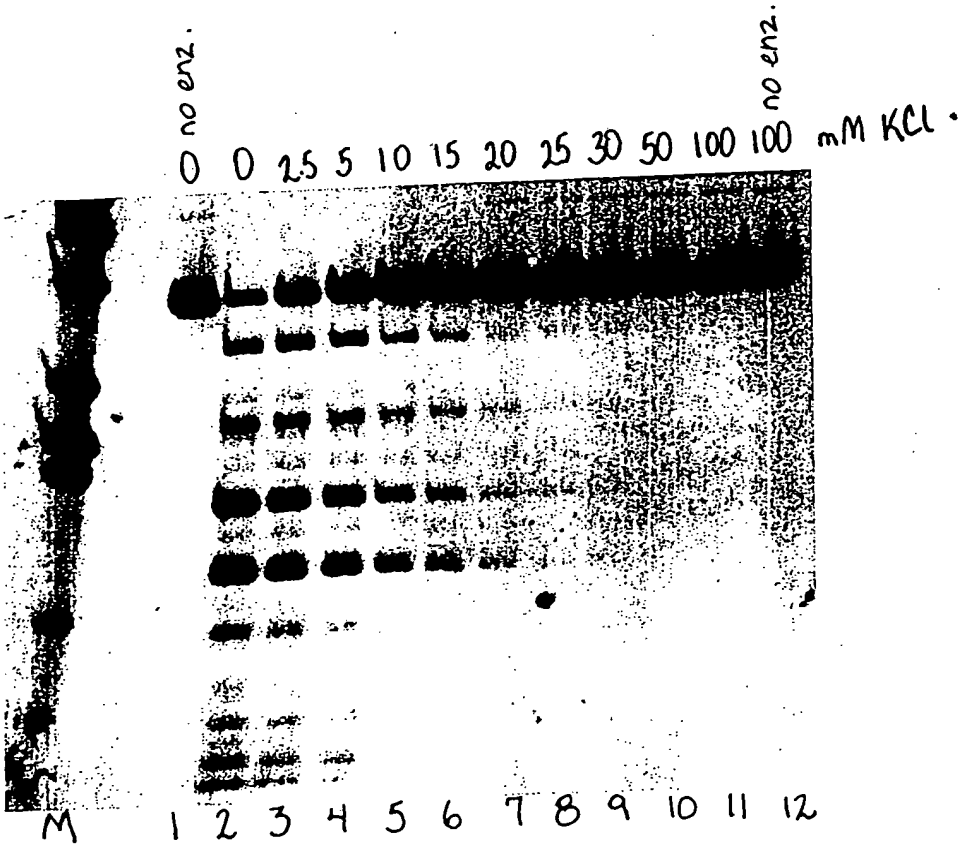

no enz.
no enz.
0 0 2.5 5 10 15 20 25 30 50 100 100 mM KCl
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 58

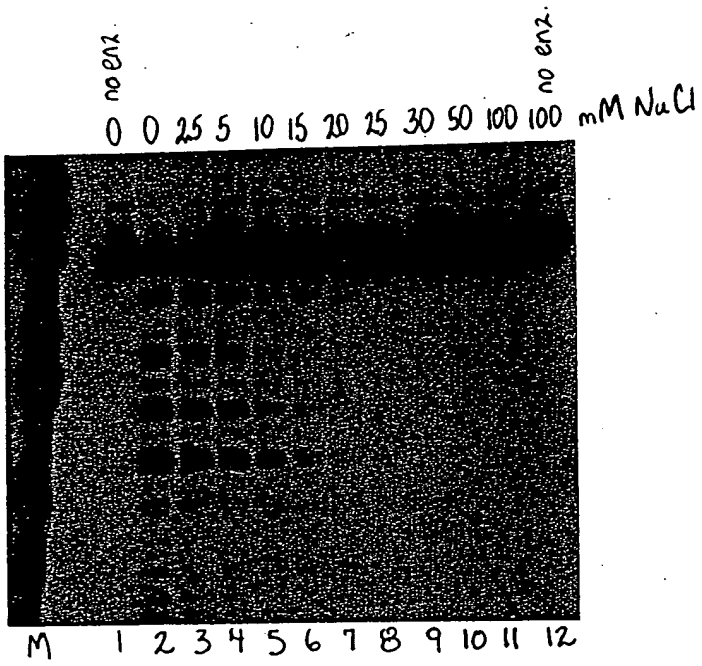

no enz.
no enz.
0 0 2.5 5 10 15 20 25 30 50 100 100 mM NaCl
M 1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 59

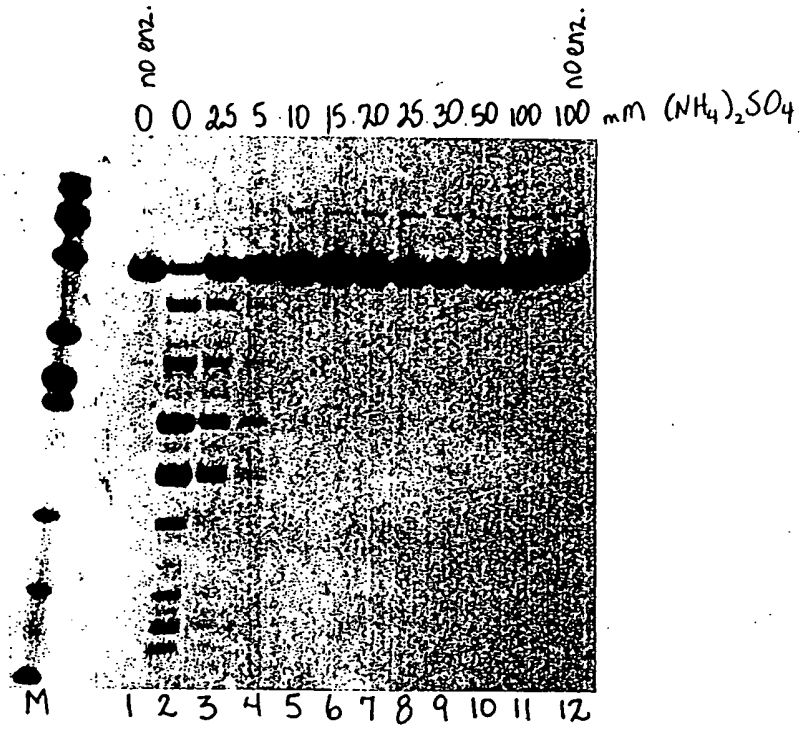

no enz.
no enz.
0 0 25 5 10 15 20 25 30 50 100 100 mM $(NH_4)_2SO_4$
M
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 60

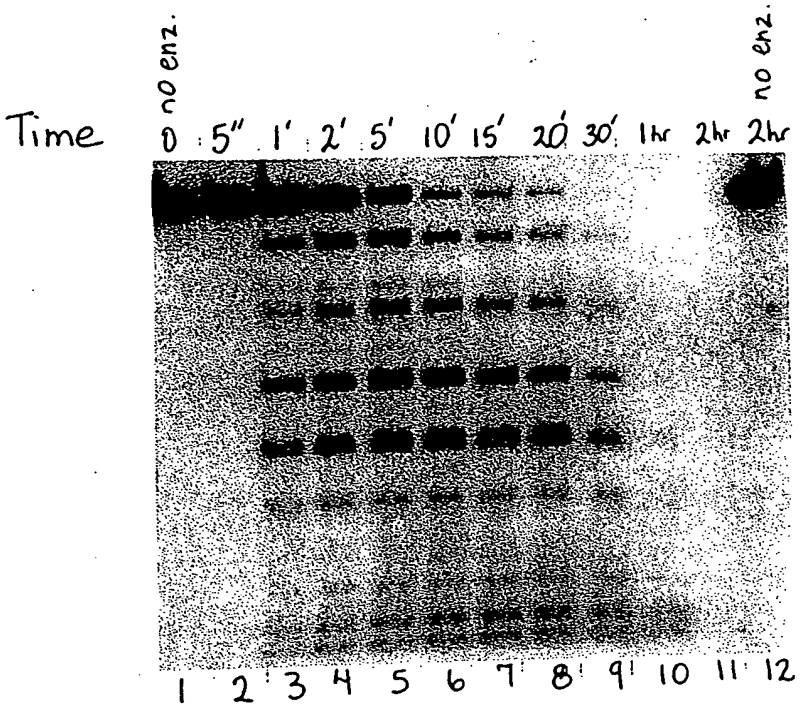

Time
no enz.
0
5"
1'
2'
5'
10'
15'
20'
30'
1hr
2hr
2hr
no enz.
1
2
3
4
5
6
7
8
9
10
11
12

FIGURE 61

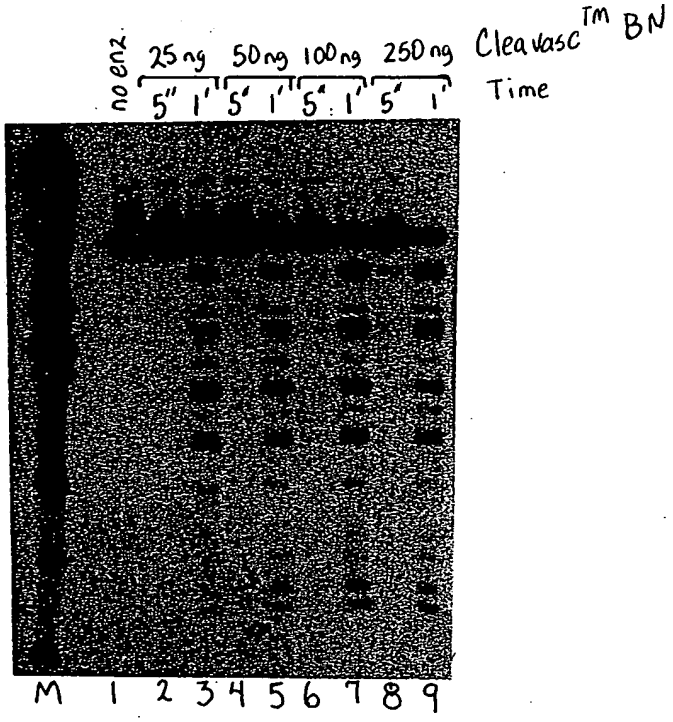

no enz
25 ng
50 ng
100 ng
250 ng
Clearasc™ BN
5" 1' 5' 1' 5' 1' 5' 1'
Time
M 1 2 3 4 5 6 7 8 9

FIGURE 62

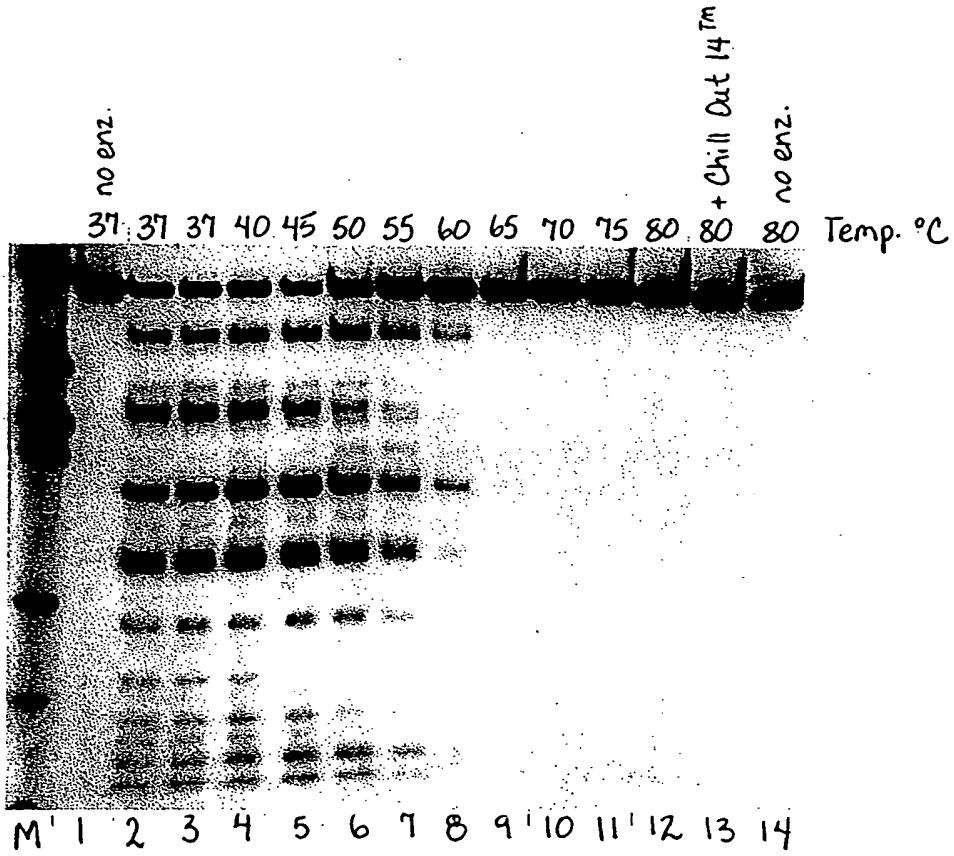

no enz.
37 37 37 40 45 50 55 60 65 70 75 80 80 80 Temp. °C
+ Chill Out 14™
no enz.
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE 63

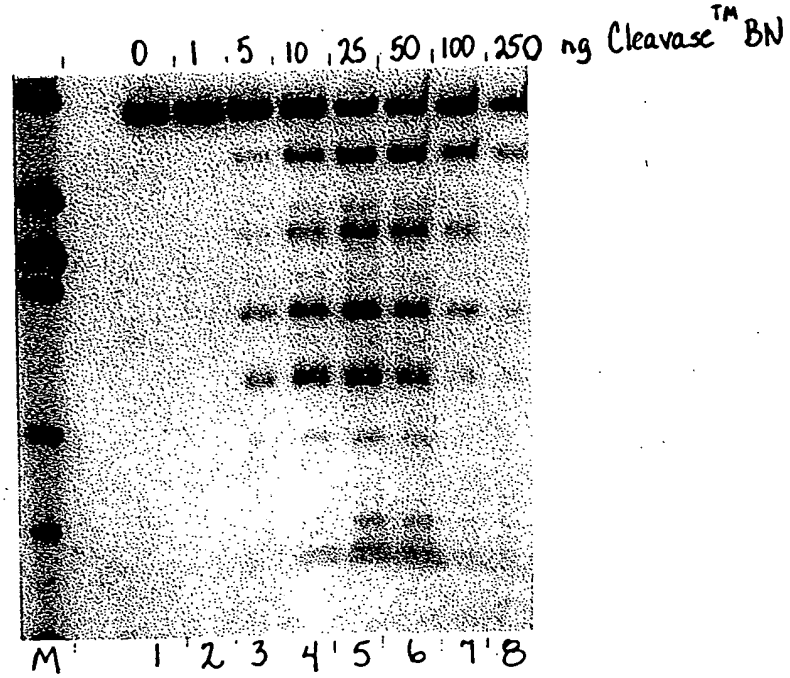
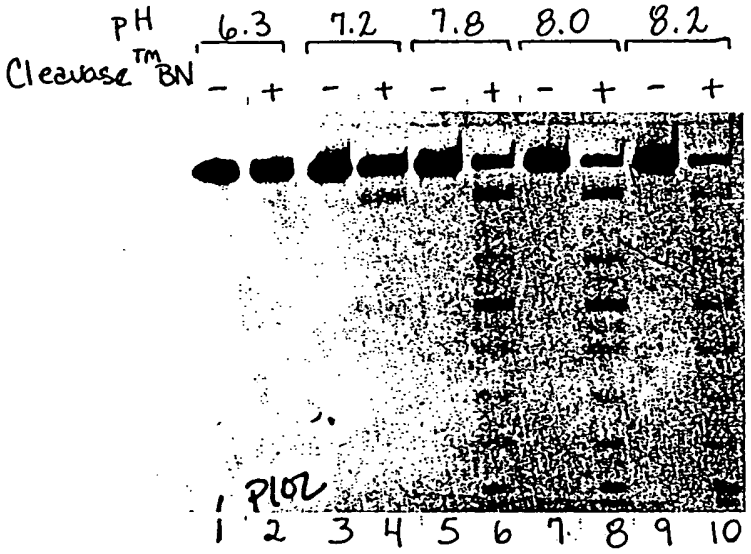


FIGURE 64

A


pH
6.3
7.2
7.8
8.0
8.2
Cleavase™ BN
- + - + - + - + - +
PLO2
1 2 3 4 5 6 7 8 9 10

B

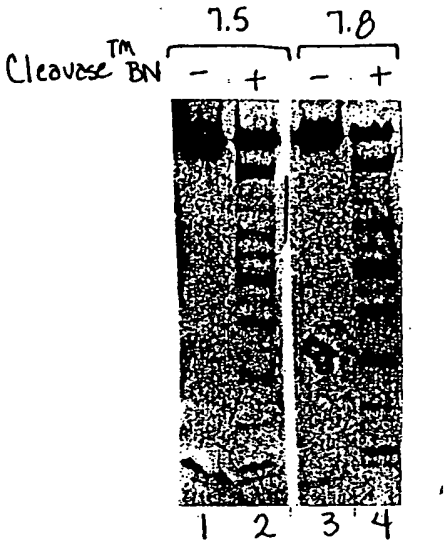

7.5
7.8
Cleavase™ BN
- + - +
1 2 3 4

FIGURE 65

A

B

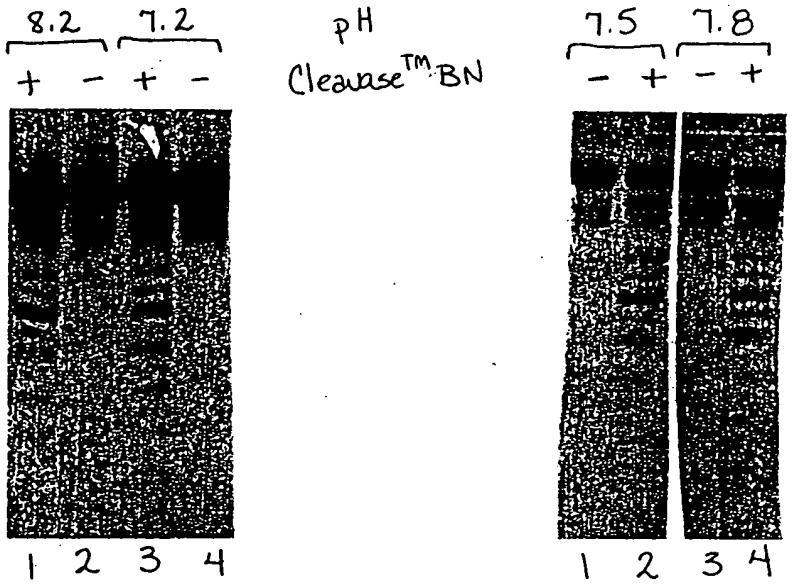


FIGURE 66

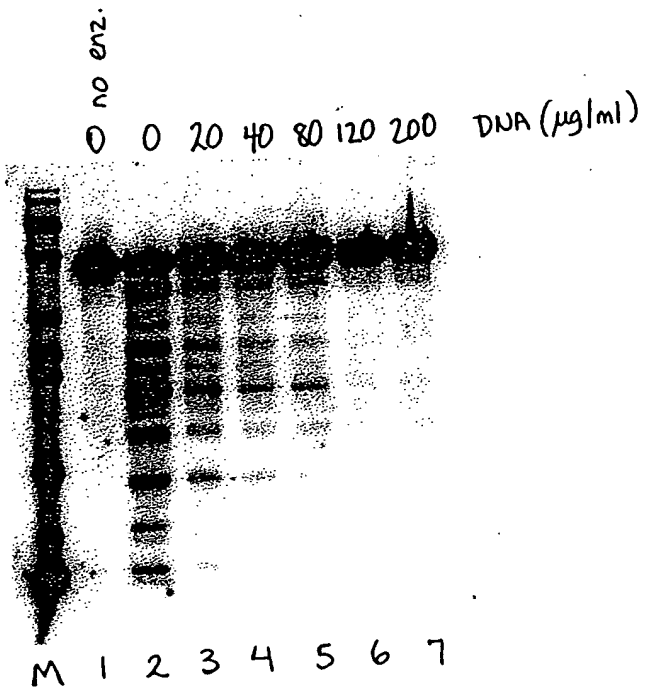


FIGURE 67

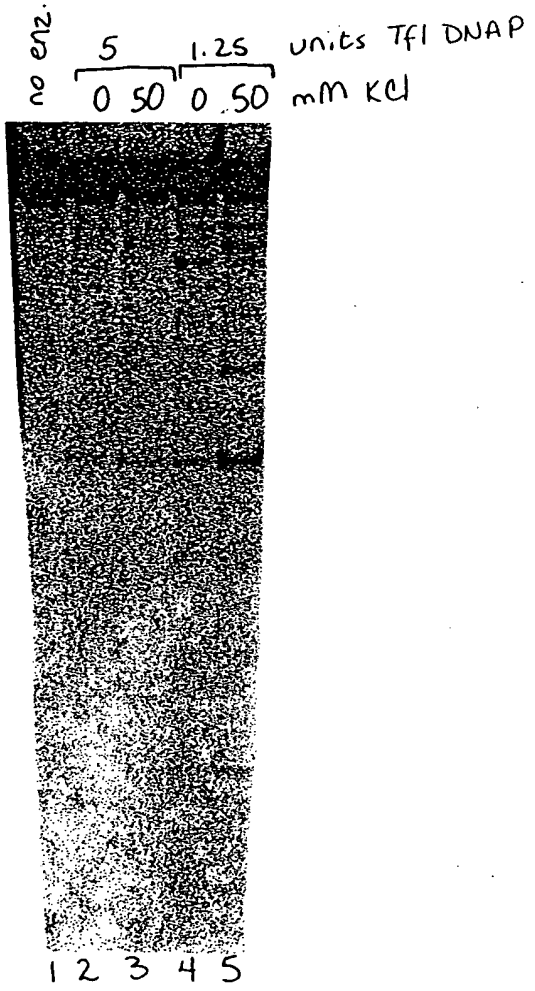


FIGURE 68

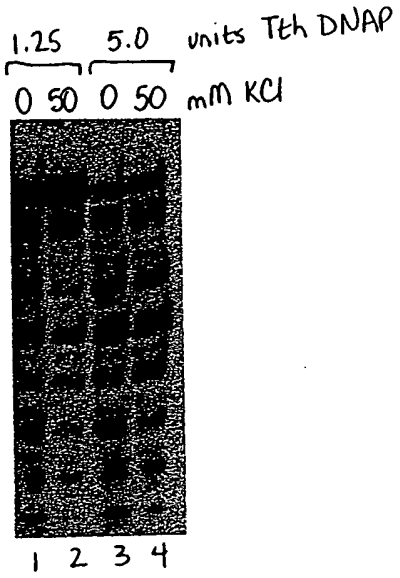


FIGURE 69

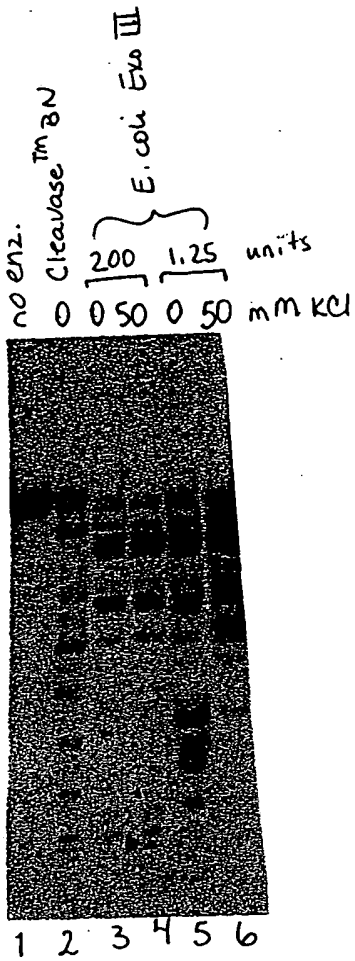

no enz.
Cleavase™ BN
E. coli Exo III
200
1.25
units
0 0 50 0 50 mM KCl
1 2 3 4 5 6

FIGURE 70

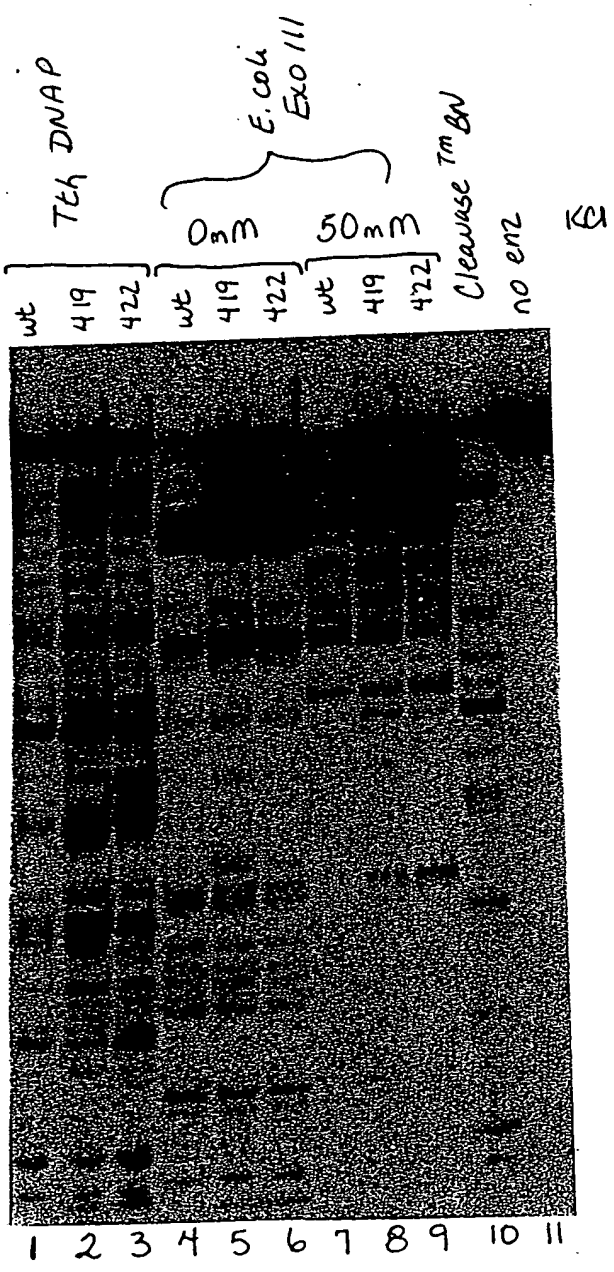
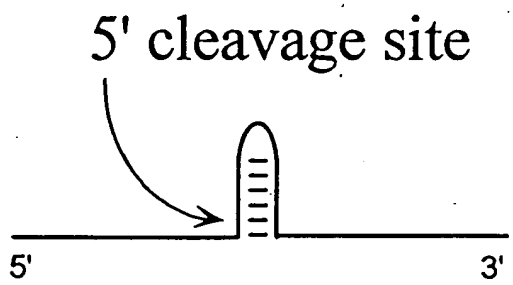

Tth DNAP
E. coli Exo III
0mM
50mM
wt
419
422
wt
419
422
wt
419
422
Cleavase™ BN
no enz
1 2 3 4 5 6 7 8 9 10 11

FIGURE 71



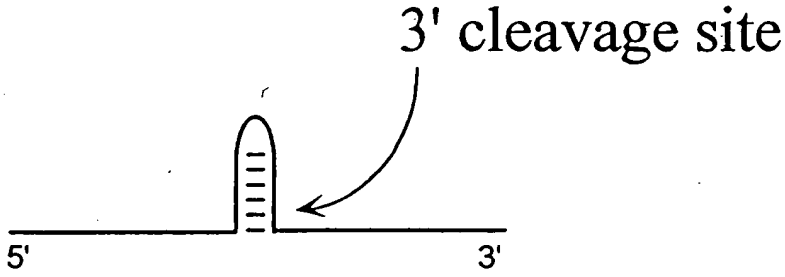


FIGURE 72

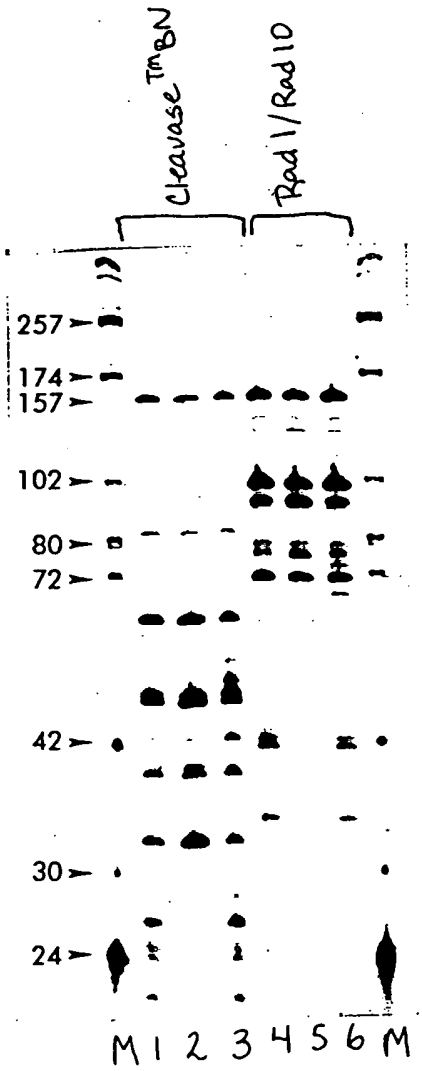
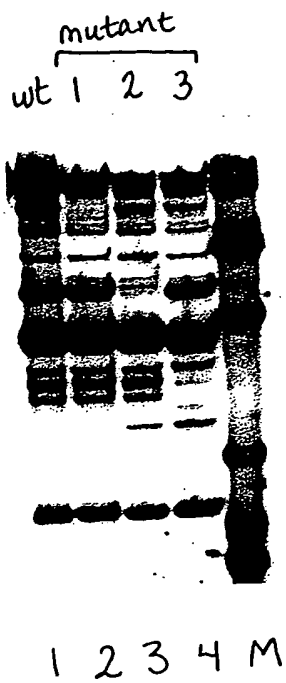


FIGURE 73

174

FIGURE 74

A



B

FIGURE 75

FIGURE 76

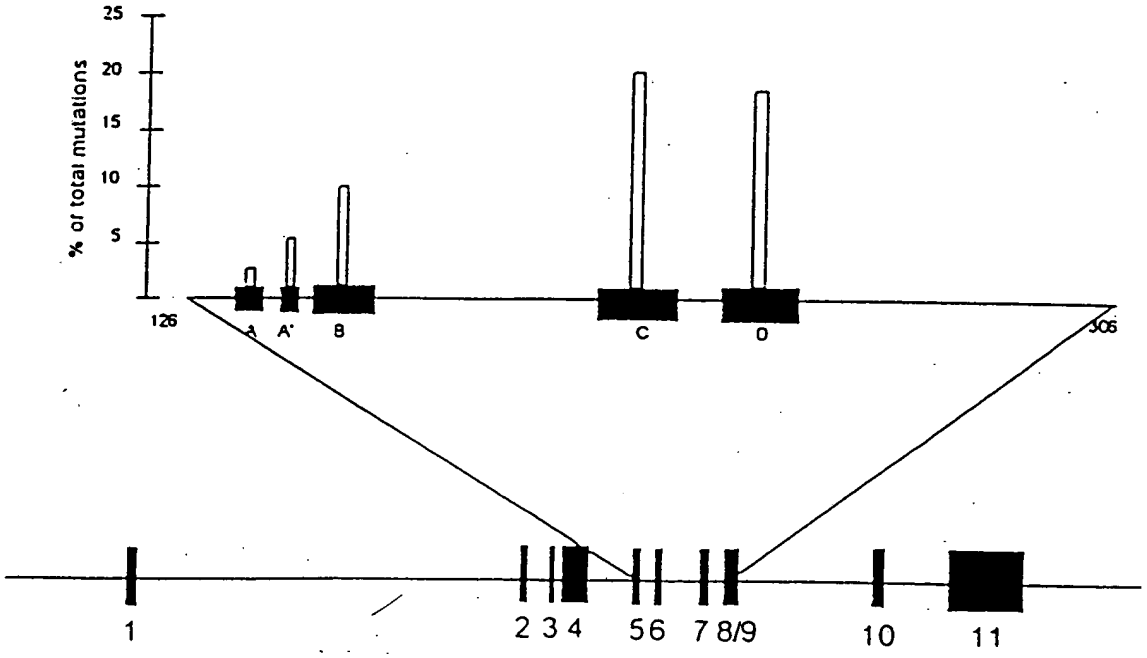


FIGURE 77

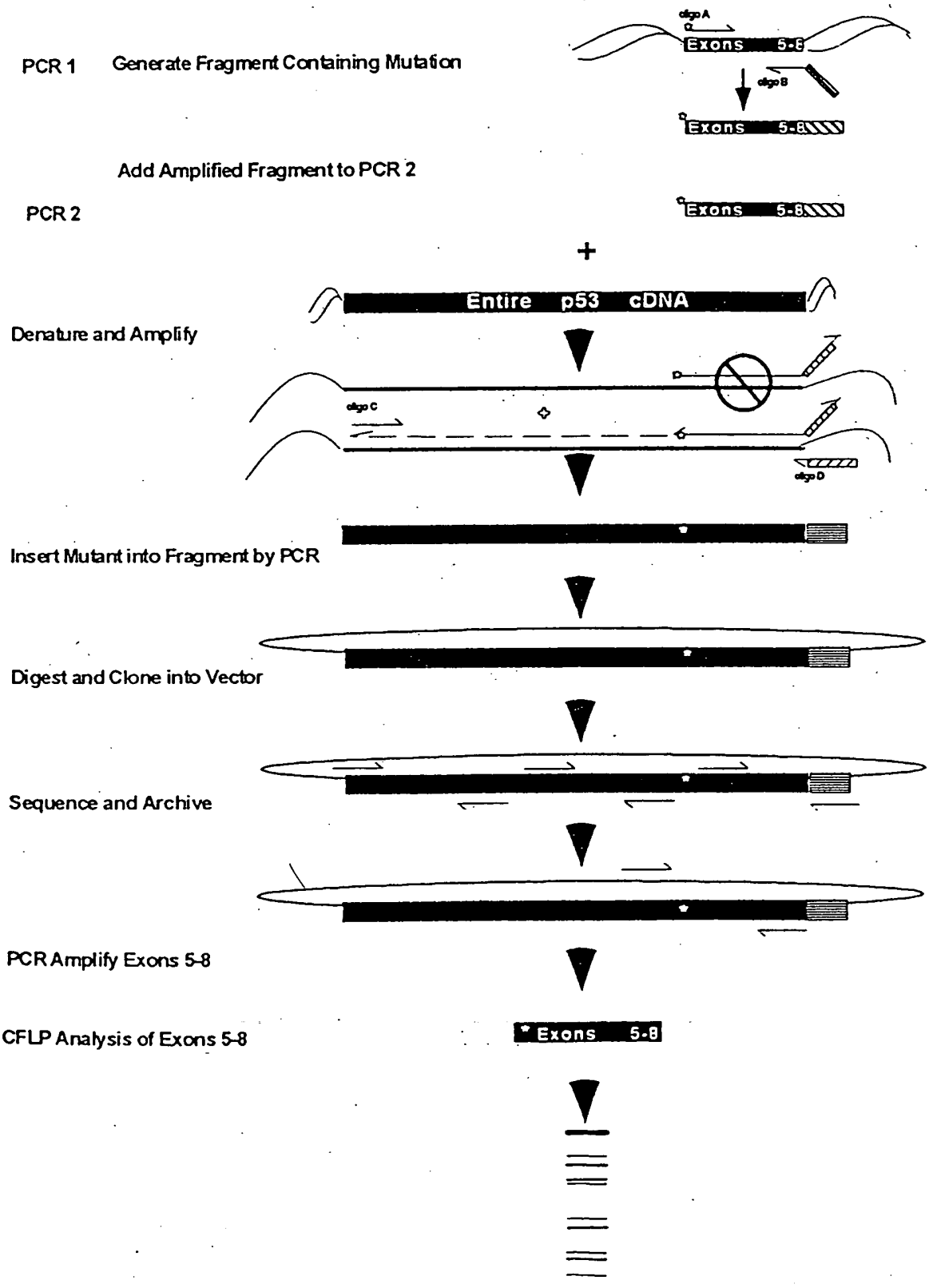

PCR 1
Generate Fragment Containing Mutation
oligo A
Exons 5-8
oligo B
Exons 5-8
Add Amplified Fragment to PCR 2
PCR 2
Exons 5-8
+
Entire p53 cDNA
Denature and Amplify
oligo C
oligo D
Insert Mutant into Fragment by PCR
Digest and Clone into Vector
Sequence and Archive
PCR Amplify Exons 5-8
CFLP Analysis of Exons 5-8
Exons 5-8

FIGURE 78

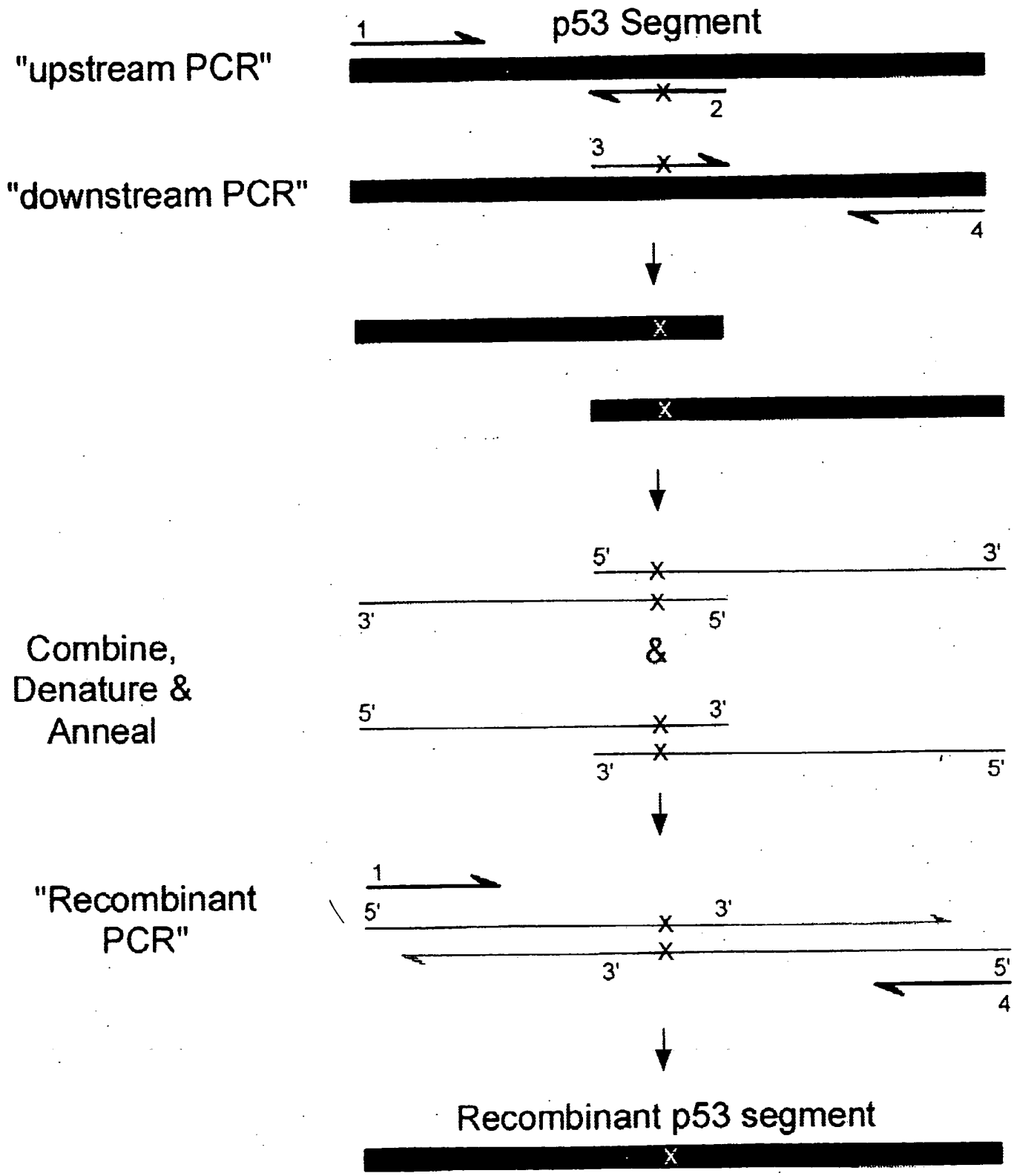

p53 Segment
1
"upstream PCR"
2
3
"downstream PCR"
4
X
X
5'
3'
3'
5'
Combine,
Denature &
Anneal
&
5'
3'
3'
5'
1
"Recombinant
PCR"
5'
3'
3'
5'
4
Recombinant p53 segment
X

FIGURE 79

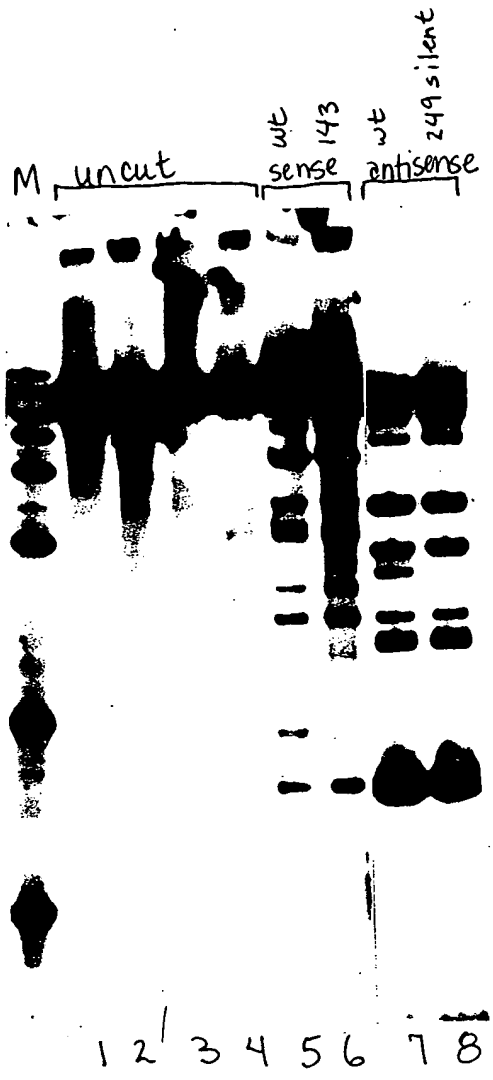


FIGURE 80

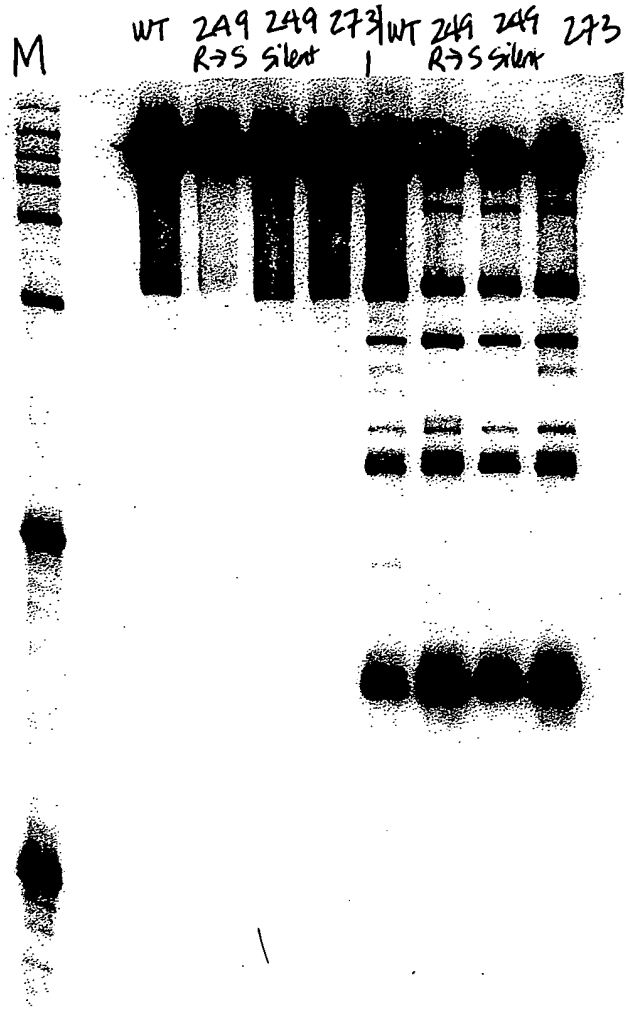


FIGURE 81

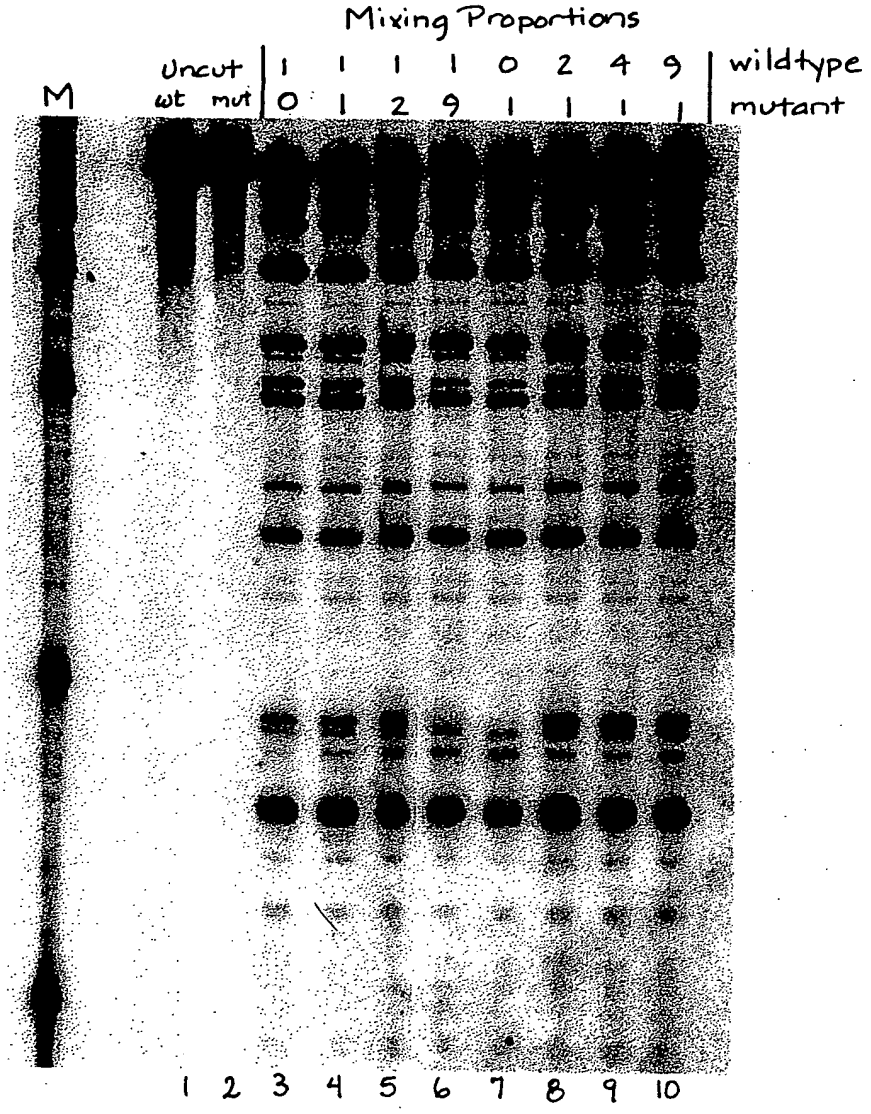

Mixing Proportions
M
Uncut
wt mut
1 1 1 1 0 2 4 9
0 1 2 9 1 1 1 1
wildtype
mutant
1 2 3 4 5 6 7 8 9 10

FIGURE 82

```
HCV1.1 (SEQ ID NO:121)      1 CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG 50
HCV2.1 (SEQ ID NO:122)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV3.1 (SEQ ID NO:123)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV4.2 (SEQ ID NO:124)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV6.1 (SEQ ID NO:125)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTA
HCV7.1 (SEQ ID NO:126)        CTGTCTTCAC GCAGAAAGCG CCTAGCCATG GCGTTAGTAC GAGTGTCGTG

HCV1.1                       51 CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC 100
HCV2.1                          CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV3.1                          CAGCCTCCAG GTCCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV4.2                          CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV6.1                          CAGCCTCCAG GCCCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV7.1                          CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC

HCV1.1                      101 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-AAA 150
HCV2.1                          GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV3.1                          GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV4.2                          GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC GTGGATGTAA
HCV6.1                          GGTGAGTACA CCGGAATTGC CGGGAAGACT GGGTCCTTTC TTGGAT-AAA
HCV7.1                          GGTGAGTACA CCGGAATCGC TGGGGTGACC GGGTCCTTTC TTGGAG-CAA

HCV1.1                      151 CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG 200
HCV2.1                          CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV3.1                          CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCGAGA CTGCTAGCCG
HCV4.2                          CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV6.1                          CCCACTCTAT GCCCGGCCAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV7.1                          CCCGCTCAAT ACCCAGAAAT TTGGGCGTGC CCCCGCGAGA TCACTAGCCG

HCV1.1                      201 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCCT 250
HCV2.1                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV3.1                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV4.2                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV6.1                          AGTAGCGTTG GGTTGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV7.1                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT

HCV1.1                      251 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC 282
HCV2.1                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV3.1                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV4.2                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV6.1                          GCGAGTACCC CGGGAGGTCT CGTAGACCGT GC
HCV7.1                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
```

FIGURE 83

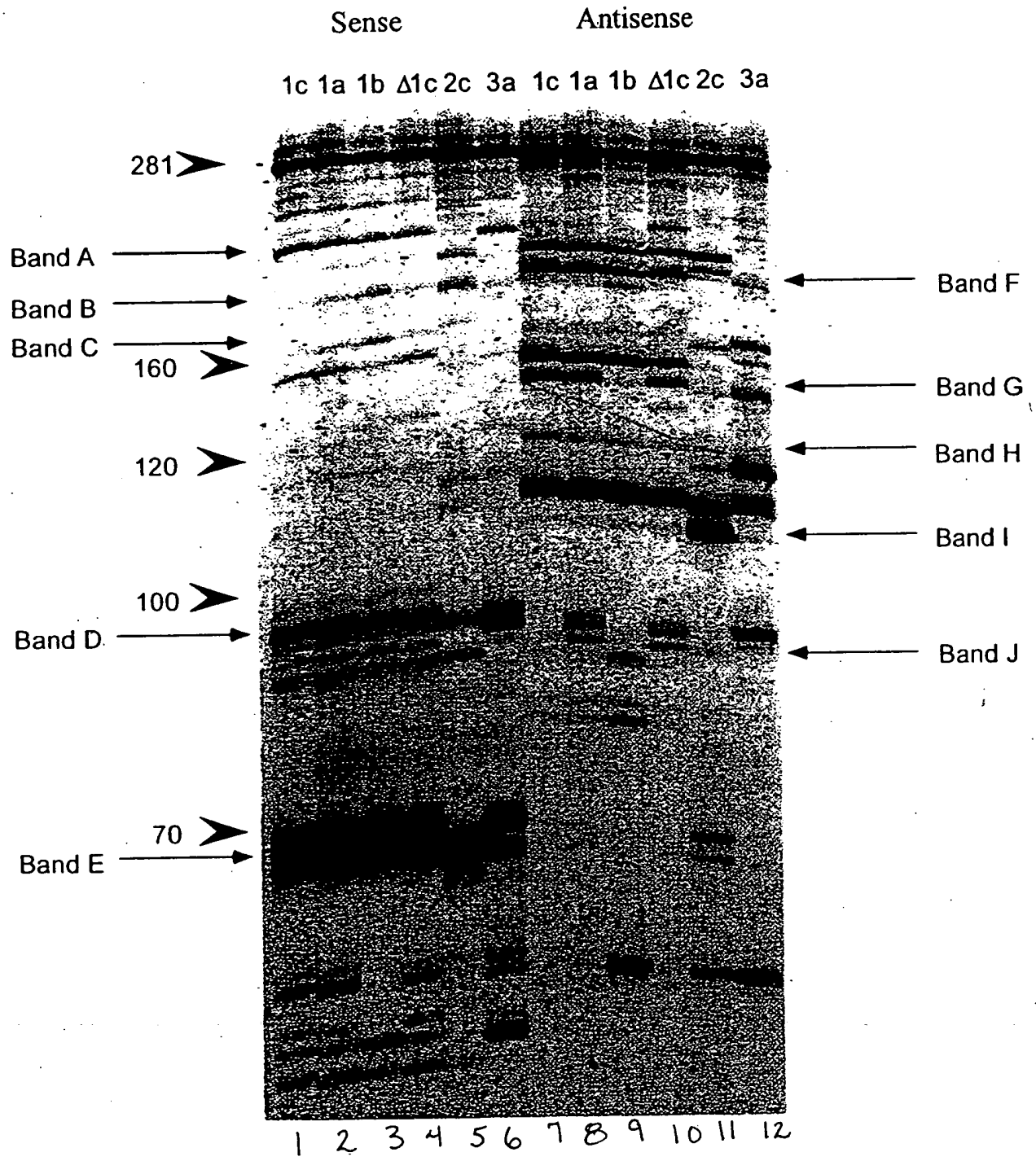

Sense
Antisense
1c 1a 1b Δ1c 2c 3a 1c 1a 1b Δ1c 2c 3a
281
Band A
Band B
Band C
160
120
100
Band D
70
Band E
Band F
Band G
Band H
Band I
Band J
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 84

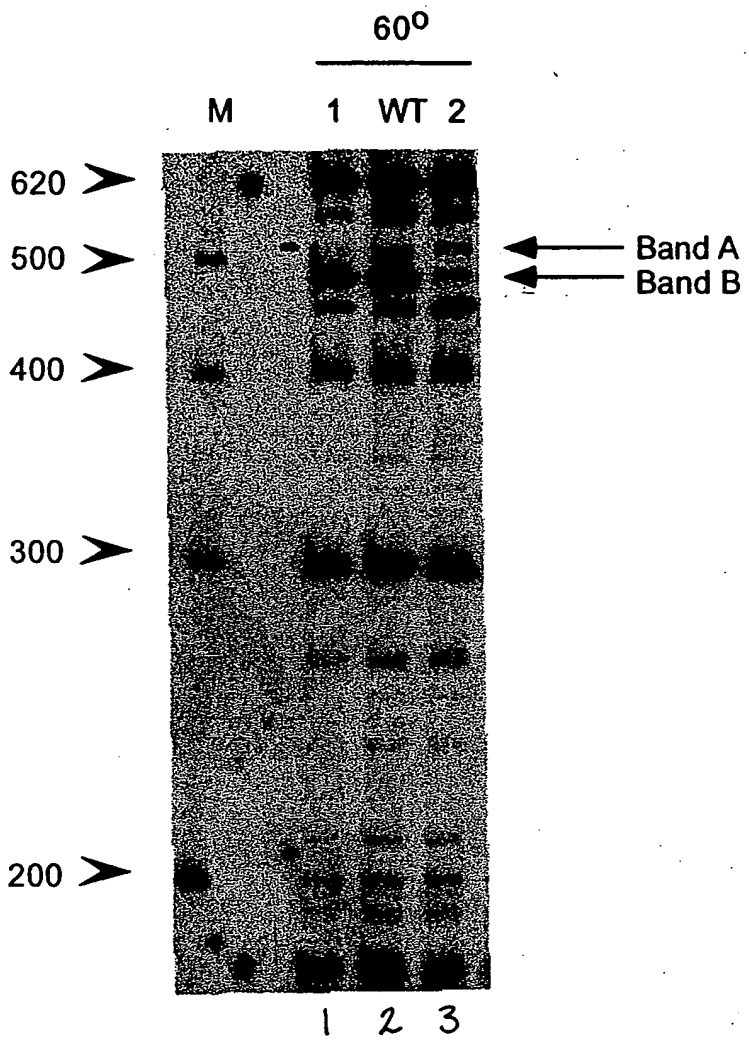


FIGURE 85

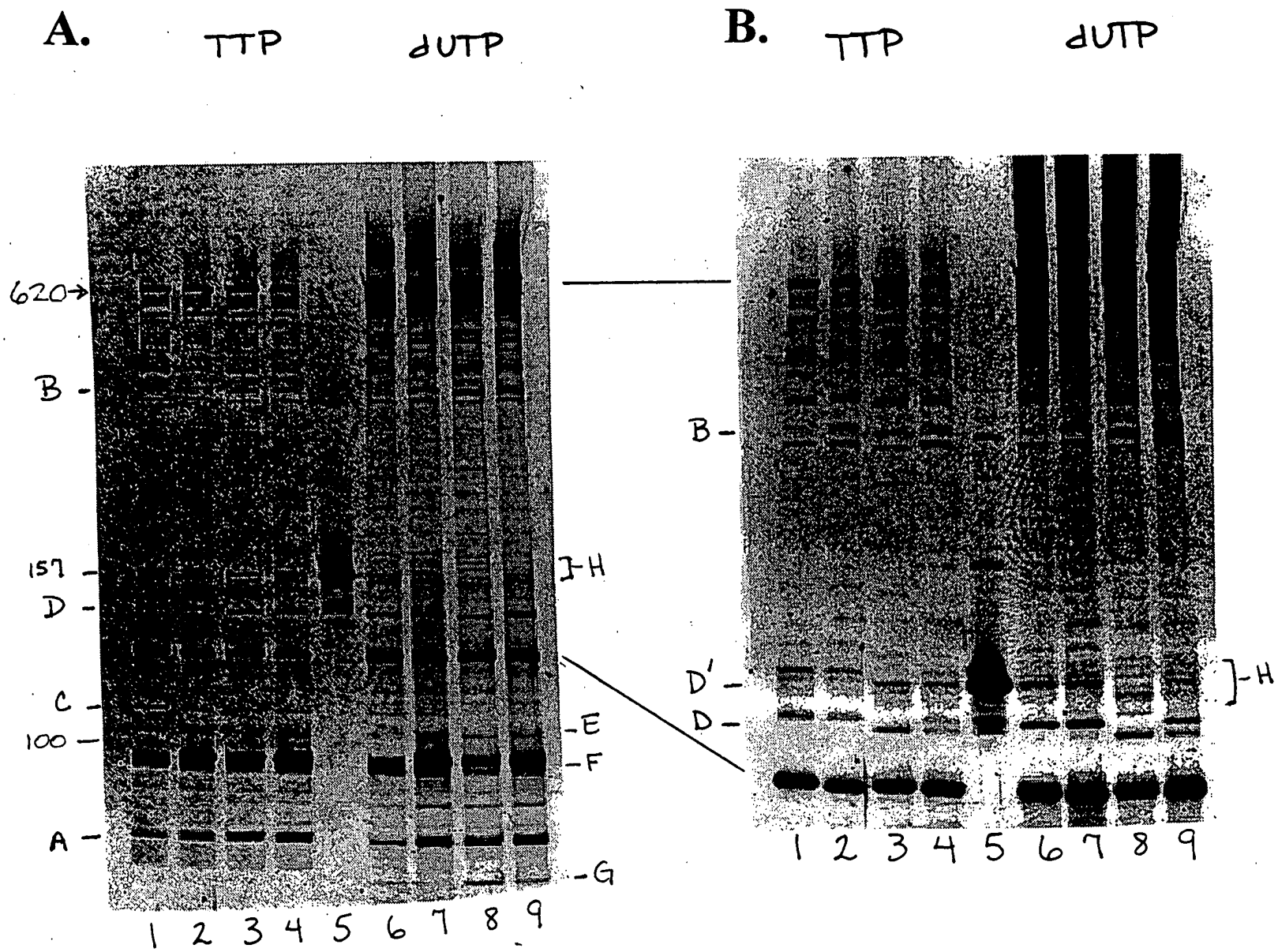

A.
TTP
dUTP
620
B
157
D
C
100
A
H
E
F
G
1 2 3 4 5 6 7 8 9
B.
TTP
dUTP
B
D'
D
H
1 2 3 4 5 6 7 8 9

FIGURE 86

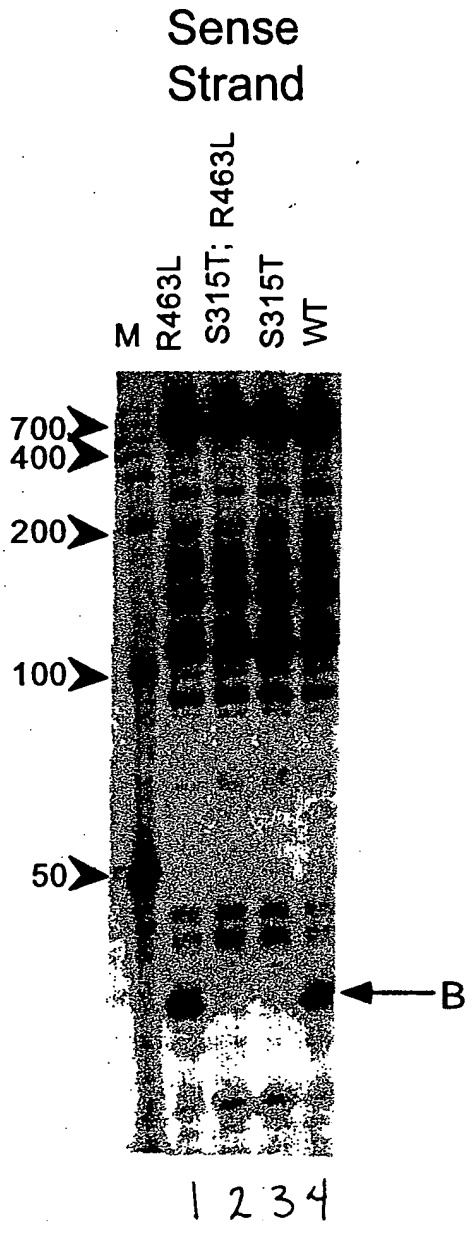
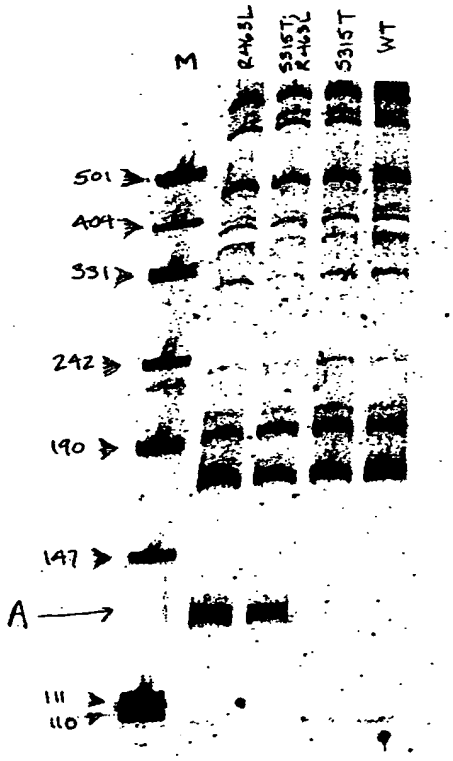

Sense
Strand
M
R463L
S315T; R463L
S315T
WT
700
400
200
100
50
B
1 2 3 4

FIGURE 87

Antisense
Strand



1 2 3 4

FIGURE 88


```
        10          20          30          40          50          60
       AGA GTTTGATCCT GGCTCAG                                          1638
AAATTGAAGA GTTTGATCAT GGCTCAGATT GAACGCTGGC GGCAGGCCTA ACACATGCAA
TTTAACTTCT CAAACTAGTA CCGAGTCTAA CTTGCGACCG CCGTCCGGAT TGTGTACGTT

        70          80          90         100         110         120
                                                GGCGGAC GGGTGAGTAA      ER10
GTCGAACGGT AACAGGAAGA AGCTTGCTTC TTTGCTGACG AGTGGCGGAC GGGTGAGTAA
CAGCTTGCCA TTGTCCTTCT TCGAACGAAG AAACGACTGC TCACCGCCTG CCCACTCATT

       130         140         150         160         170         180
TGTCTGGGAA ACTGCCTGAT GGAGGGGGAT AACTACTGGA AACGGTAGCT AATACCGCAT
ACAGACCCTT TGACGGACTA CCTCCCCCTA TTGATGACCT TTGCCATCGA TTATGGCGTA

       190         200         210         220         230         240
AACGTCGCAA GACCAAAGAG GGGGACCTTC GGGCCTCTTG CCATCGGATG TGCCCAGATG
TTGCAGCGTT CTGGTTTCTC CCCCTGGAAG CCCGGAGAAC GGTAGCCTAC ACGGGTCTAC

       250         260         270         280         290         300
GGATTAGCTA GTAGGTGGGG TAACGGCTCA CCTAGGCGAC GATCCCTAGC TGGTCTGAGA
CCTAATCGAT CATCCACCCC ATTGCCGAGT GGATCCGCTG CTAGGGATCG ACCAGACTCT

       310         320         330         340         350         360
GGATGACCAG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG GCAGCAGTGG
CCTACTGGTC GGTGTGACCT TGACTCTGTG CCAGGTCTGA GGATGCCCTC CGTCGTCACC
                                  TGA GGATGCCCTC CGTCGTC             1659

       370         380         390         400         410         420
GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC CGCGTGTATG AAGAAGGCCT
CCTTATAACG TGTTACCCGC GTTCGGACTA CGTCGGTACG GCGCACATAC TTCTTCCGGA

       430         440         450         460         470         480
TCGGGTTGTA AAGTACTTTC AGCGGGGAGG AAGGGAGTAA AGTTAATACC TTTGCTCATT
AGCCCAACAT TTCATGAAAG TCGCCCCTCC TTCCCTCATT TCAATTATGG AAACGAGTAA

       490         500         510         520         530         540
GACGTTACCC GCAGAAGAAG CACCGGCTAA CTCCGTGCCA GCAGCCGCGG TAATACGGAG
CTGCAATGGG CGTCTTCTTC GTGGCCGATT GAGGCACGGT CGTCGGCGCC ATTATGCCTC

       550         560         570         580         590         600
GGTGCAAGCG TTAATCGGAA TTACTGGGCG TAAAGCGCAC GCAGGCGGTT TGTTAAGTCA
CCACGTTCGC AATTAGCCTT AATGACCCGC ATTTCGCGTG CGTCCGCCAA ACAATTCAGT

       610         620         630         640         650         660
GATGTGAAAT CCCCGGGCTC AACCTGGGAA CTGCATCTGA TACTGGCAAG CTTGAGTCTC
CTACACTTTA GGGGCCCGAG TTGGACCCTT GACGTAGACT ATGACCGTTC GAACTCAGAG

       670         680         690         700         710         720
GTAGAGGGGG GTAGAATTCC AGGTGTAGCG GTGAAATGCG TAGAGATCTG GAGGAATACC
CATCTCCCCC CATCTTAAGG TCCACATCGC CACTTTACGC ATCTCTAGAC CTCCTTATGG

       730         740         750         760         770         780
GGTGGCGAAG GCGGCCCCCT GGACGAAGAC TGACGCTCAG GTGCGAAAGC GTGGGGAGCA
CCACCGCTTC CGCCGGGGGA CCTGCTTCTG ACTGCGAGTC CACGCTTTCG CACCCCTCGT
```

```
      790         800         810         820         830         840
AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGAT GTCGACTTGG AGGTTGTGCC
TTGTCCTAAT CTATGGGACC ATCAGGTGCG GCATTTGCTA CAGCTGAACC TCCAACACGG

      850         860         870         880         890         900
CTTGAGGCGT GGCTTCCGGA GCTAACGCGT TAAGTCGACC GCCTGGGGAG TACGGCCGCA
GAACTCCGCA CCGAAGGCCT CGATTGCGCA ATTCAGCTGG CGGACCCCTC ATGCCGGCGT

      910         920         930         940         950         960
AGGTTAAAAC TCAAATGAAT TGACGGGGGC CCGCACAAGC GGTGGAGCAT GTGGTTTAAT
TCCAATTTTG AGTTTACTTA ACTGCCCCCG GGCGTGTTCG CCACCTCGTA CACCAAATTA

      970         980         990        1000        1010        1020
TCGATGCAAC GCGAAGAACC TTACCTGGTC TTGACATCCA CGGAAGTTTT CAGAGATGAG
AGCTACGTTG CGCTTCTTGG AATGGACCAG AACTGTAGGT GCCTTCAAAA GTCTCTACTC

     1030        1040        1050        1060        1070        1080
AATGTGCCTT CGGGAACCGT GAGACAGGTG CTGCATGGCT GTCGTCAGCT CGTGTTGTGA
TTACACGGAA GCCCTTGGCA CTCTGTCCAC GACGTACCGA CAGCAGTCGA GCACAACACT

     1090        1100        1110        1120        1130        1140
                   GC AACGAGCGCA ACCC                                    SB-1
AATGTTGGGT TAAGTCCCGC AACGAGCGCA ACCCTTATCC TTTGTTGCCA GCGGTCCGGC
TTACAACCCA ATTCAGGGCG TTGCTCGCGT TGGGAATAGG AAACAACGGT CGCCAGGCCG

     1150        1160        1170        1180        1190        1200
                                                  ATG ACGTCAAGTC       SB-3
                                                  ATG ACGTCAAGTC       SB-4
CGGGAACTCA AAGGAGACTG CCAGTGATAA ACTGGAGGAA GGTGGGGATG ACGTCAAGTC
GCCCTTGAGT TTCCTCTGAC GGTCACTATT TGACCTCCTT CCACCCCTAC TGCAGTTCAG

     1210        1220        1230        1240        1250        1260
                                                                       SB-3
ATCATGGCCC TTA                                                         SB-4
ATCATGGCCC TTACGA
ATCATGGCCC TTACGACCAG GGCTACACAC GTGCTACAAT GGCGCATACA AAGAGAAGCG
TAGTACCGGG AATGCTGGTC CCGATGTGTG CACGATGTTA CCGCGTATGT TTCTCTTCGC

     1270        1280        1290        1300        1310        1320
ACCTCGCGAG AGCAAGCGGA CCTCATAAAG TGCGTCGTAG TCCGGATTGG AGTCTGCAAC
TGGAGCGCTC TCGTTCGCCT GGAGTATTTC ACGCAGCATC AGGCCTAACC TCAGACGTTG

     1330        1340        1350        1360        1370        1380
TCGACTCCAT GAAGTCGGAA TCGCTAGTAA TCGTGGATCA GAATGCCACG GTGAATACGT
AGCTGAGGTA CTTCAGCCTT AGCGATCATT AGCACCTAGT CTTACGGTGC CACTTATGCA
                                                  GC CACTTATGCA        1743

     1390        1400        1410        1420        1430        1440
TCCCGGGCCT TGTACACACC GCCCGTCACA CCATGGGAGT GGGTTGCAAA AGAAGTAGGT
AGGGCCCGGA ACATGTGTGG CGGGCAGTGT GGTACCCTCA CCCAACGTTT TCTTCATCCA
                                                                       1743
AGGGCCCGGA ACATG

     1450        1460        1470        1480        1490        1500
AGCTTAACCT TCGGGAGGGC GCTTACCACT TTGTGATTCA TGACTGGGGT GAAGTCGTAA
TCGAATTGGA AGCCCTCCCG CGAATGGTGA AACACTAAGT ACTGACCCCA CTTCAGCATT

     1510        1520        1530        1540        1550
CAAGGTAACC GTAGGGGAAC CTGCGGTTGG ATCACCTCCT TA.......
GTTCCATTGG CATCCCCTTG GACGCCAACC TAGTGGAGGA AT.......
```

FIGURE 89


```
1638 (SEQ ID NO:151)                    AGAGTTTGATCCTGGCTCAG
E.colirrsE (SEQ ID NO:158)0 ...AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCA
Cam.jejun5 (SEQ ID NO:159)0 -TTTTTATGGAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCGTGCCTAATACATGCA
Stp.aureus (SEQ ID NO:160)0 ..TTTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA

                                                                        GGCGGACGGG
ER10 (SEQ ID NO:152)
E.colirrsE                  60 AGTCGAACGGTAACAG----GAAGAAGCTTGCTTCTTT----GCTGACGAGTGGCGGACGGG
Cam.jejun5                  62 AGTCGAACGAT-----GAAGCTTCTAGCTTGCTAGAAGTGGA-----TTAGTGGCGCACGGG
Stp.aureus                  61 AGTCGAGCGAA-----CGGACGAGAAGCTTGCTTCTCTGATG----TT-AGCGGCGGACGGG

ER10                           TGAGTAA
E.colirrsE                 114 TGAGTAATGTCTGGGA-AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA
Cam.jejun5                 114 TGAGTAAGGTATAGTTAATCTGCCCTACACAAGAGGACAACAGTTGGAAACGACTGCTAATA
Stp.aureus                 113 TGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATA

E.colirrsE                 175 CCGCATAAC-------GTCGCAAGAC---------CAAAGAGGGGGACCTTCG-GGCCTCTTG
Cam.jejun5                 176 CTCTATACTCCTGCTTAACACAAGTTGAGTAGG-GAAAG---------TTTTT-------CG
Stp.aureus                 175 CCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGT----CTT----GCTGTCA

E.colirrsE                 221 CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGA
Cam.jejun5                 221 GTGTAGGATGAGACTATATAGTATCAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCTATGA
Stp.aureus                 229 CTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGA

E.colirrsE                 283 TCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
Cam.jejun5                 283 CGCTTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTA
Stp.aureus                 291 TACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
1659(COMPL)                                                             ACTCCTA

E.colirrsE                 345 CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTG
Cam.jejun5                 345 CGGGAGGCAGCAGTAGGGAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTG
Stp.aureus                 353 CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTG
1659(COMPL)                    CGGGAGGCAGCAG

E.colirrsE                 407 TATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA-GGGAGTAAAGTTAAT
Cam.jejun5                 407 GAGGATGACACTTTTCGGAGCGTAAACTCCTTTTCTTAGGGAAG--------------AATT
Stp.aureus                 415 AGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAAC

E.colirrsE                 468 ACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Cam.jejun5                 455 C-----------TGACGGTACCTAAGGAATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Stp.aureus                 476 -TGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG
```

```
E.colirrsE   530 GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTT
Cam.jejun5   506 GTAATACGGAGGGTGCAAGCGTTACTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGATT
Stp.aureus   538 GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTT

E.colirrsE   592 GTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTT
Cam.jejun5   568 ATCAAGTCTCTTGTGAAATCTAATGGCTTAACCATTAAACTGCTTGGGAAACTGATAGTCTA
Stp.aureus   600 TTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTT

E.colirrsE   654 GAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA
Cam.jejun5   630 GAGTGAGGGAGAGGCAGATGGAATTGGTGGTGTAGGGGTAAAATCCGTAGATATCACCAAGA
Stp.aureus   662 GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGA

E.colirrsE   716 ATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA
Cam.jejun5   692 ATACCCATTGCGAAGGCGATCTGCTGGAACTCAACTGACGCTAAGGCGCGAAAGCGTGGGGA
Stp.aureus   724 ACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGA

E.colirrsE   778 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGC
Cam.jejun5   754 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTACACTAGTTGTTGGGGT
Stp.aureus   786 TCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGG

E.colirrsE   840 C-CTTGA-GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGC
Cam.jejun5   816 G-CTAGT-CATCTCAGTAATGCAGCTAACGCATTAAGTGTACCGCCTGGGGAGTACGGTCGC
Stp.aureus   848 GT-TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGC

E.colirrsE   900 AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Cam.jejun5   876 AAGATTAAAACTCAAAGGAATAGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Stp.aureus   909 AAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT

E.colirrsE   962 CGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAAT
Cam.jejun5   938 CGAAGATACGCGAAGAACCTTACCTGGGCTTGATATCCTAAGAACCTTTTAGAGATAAGAGG
Stp.aureus   971 CGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCC

E.colirrsE  1024 GTG--CCTTCGGG--AA-CCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
Cam.jejun5  1000 GTGCTAGCTTGCTAGAA-CTTAGAGACAGGTGCTGCACGGCTGTCGTCAGCTCGTGTCGTGA
Stp.aureus  1033 TTCC-CCTTCGGG--GGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA

SB-1                                GCAACGAGCGCAACCC
E.colirrsE  1081 AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGG-CC
Cam.jejun5  1061 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTATTTAGTTGCTAACGGTTCGG-CC
Stp.aureus  1092 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCA-TTAAGT-T
```

FIGURE 89


```
SB-3  (SEQ ID NO:157)                                                ATGACGTCAAGTCATC
SB-4  (SEQ ID NO:154)                                                ATGACGTCAAGTCATC
E.colirrsE 1142 GGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATC
Cam.jejun5 1122 GAGCACTCTAAATAGACTGCCTTCG-TAAGGAGGAGGAAGGTGTGGACGACGTCAAGTCATC
Stp.aureus 1152 GGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC

SB-3            ATGGCCCTTA
SB-4            ATGGCCCTTACGA
E.colirrsE 1204 ATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTC
Cam.jejun5 1183 ATGGCCCTTATGCCCAGGGCGACACACGTGCTACAATGGCATATAGAATGAGACGCAATACC
Stp.aureus 1214 ATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACC

E.colirrsE 1266 GCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTC
Cam.jejun5 1245 GCGAGGTGGAG-CAAATCTATAAAATATGTCCCAGTTCGGATTGTTCTCTGCAACTCGAGAG
Stp.aureus 1276 GCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTA

E.colirrsE 1328 CATGAAGTCGGAATCGCTAGTAATCGTGGATCAGA-ATGCCACGGTGAATACGTTCCCGGGC
Cam.jejun5 1306 CATGAAGCCGGAATCGCTAGTAATCGTAGATCAGCCATGCTACGGTGAATACGTTCCCGGGT
Stp.aureus 1338 CATGAAGCTGGAATCGCTAGTAATCGTAGATCAGC-ATGCTACGGTGAATACGTTCCCGGGT
1743(compl)                                                          CGGTGAATACGTTCCCGGGC

E.colirrsE 1389 CTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCT
Cam.jejun5 1368 CTTGTACTCACCGCCCGTCACACCATGGGAGTTGATTTCACTCGAAGCCGGAATACT--A-A
Stp.aureus 1399 ATTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCT
1743(compl)     CTTGTAC

E.colirrsE 1451 TCG-GGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCG
Cam.jejun5 1427 AC---T-AGTTACCGTCCACAGTGGAATCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCG
Stp.aureus 1461 TTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGTGAAGTCGTAACAAGGTAGCCG

E.colirrsE 1512 TAGGGGAACCTGCGGTTGGATCACCTCCTTA---
Cam.jejun5 1485 TAGGAGAACCTGCGGTTGGATCACCTCCT-----
Stp.aureus 1523 TATCGGAAGGTGCGGCTGGATCACCTCCTTTCT-
```

FIGURE 90

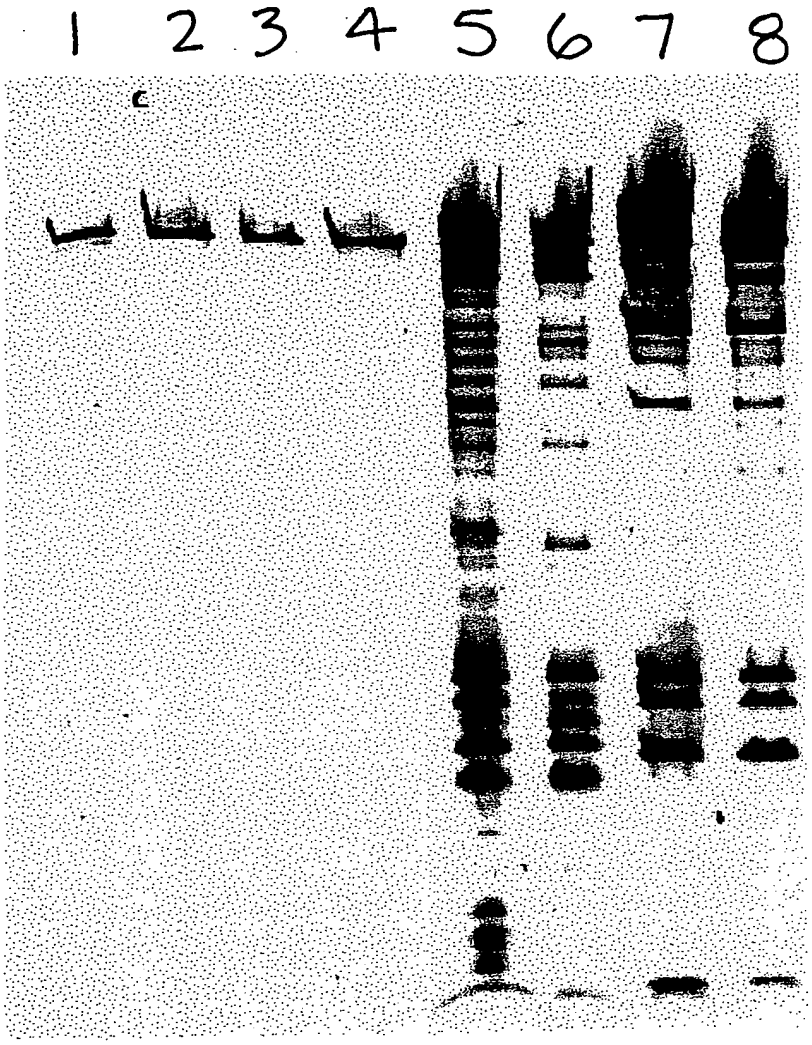
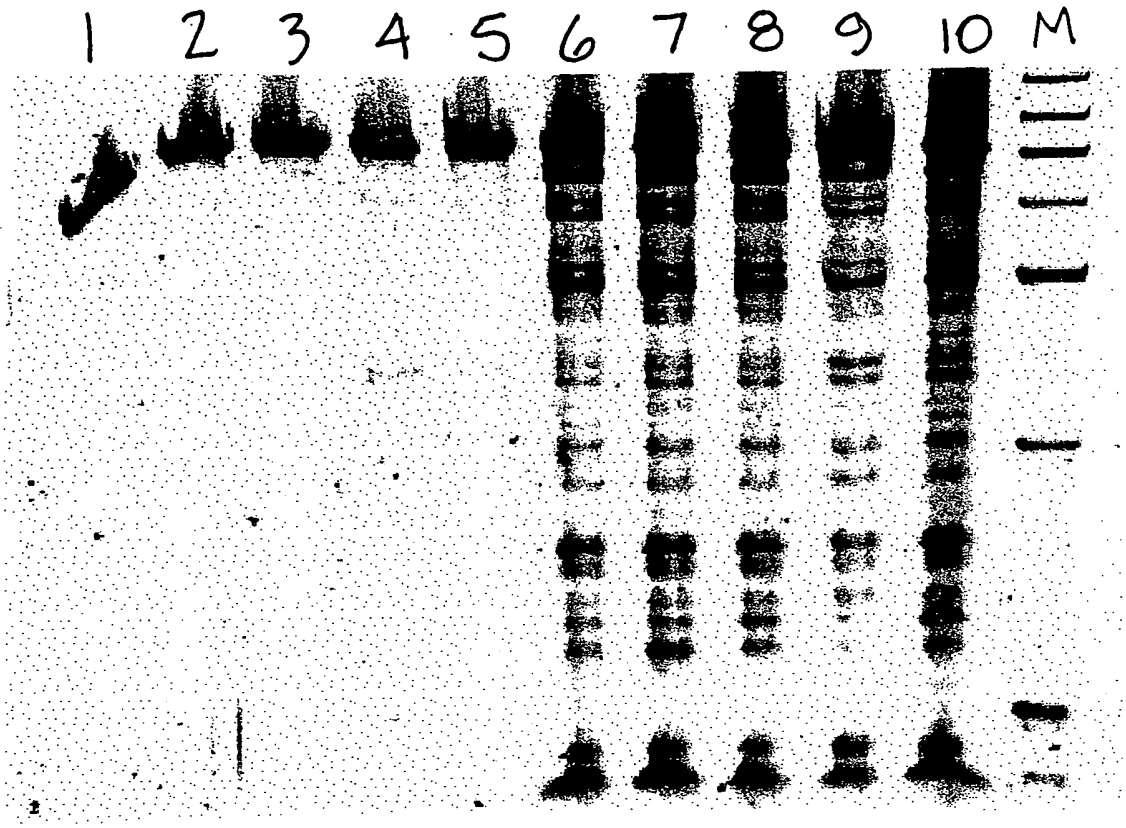


FIGURE 91

A.



B.

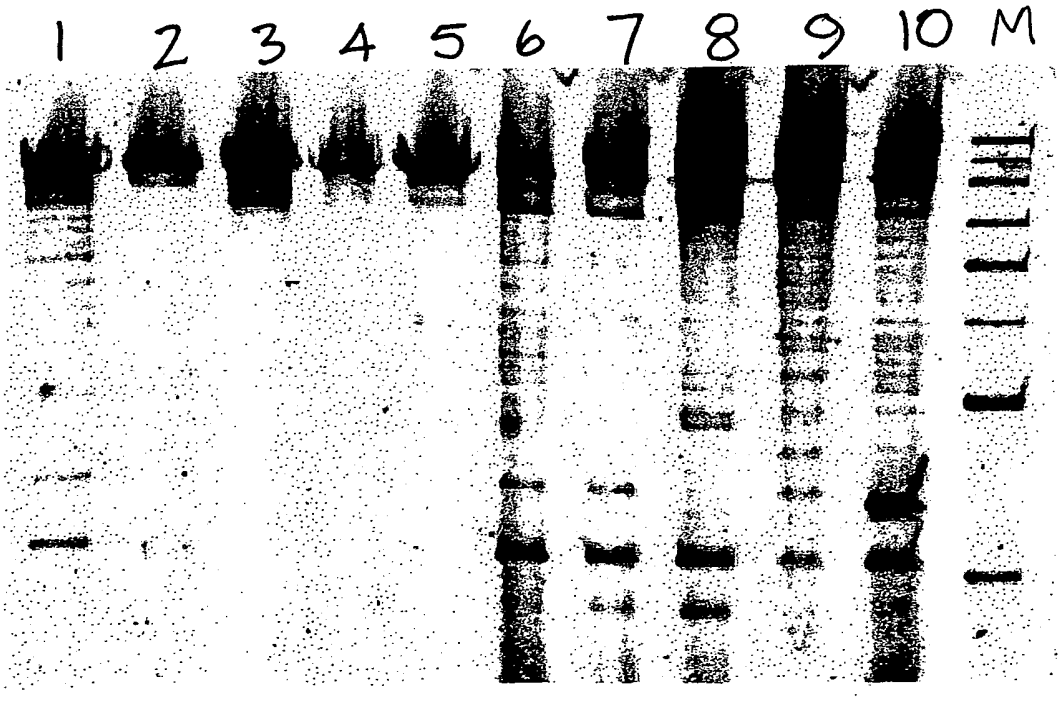


FIGURE 92

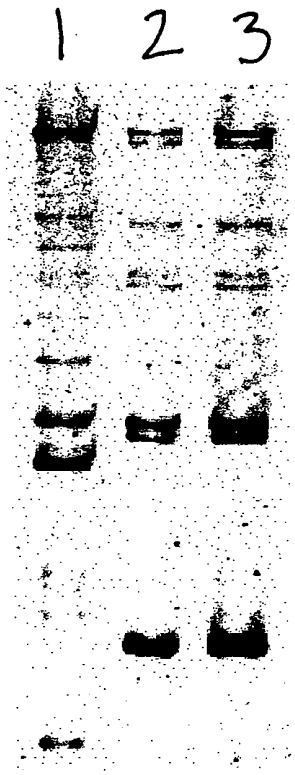


FIGURE 93

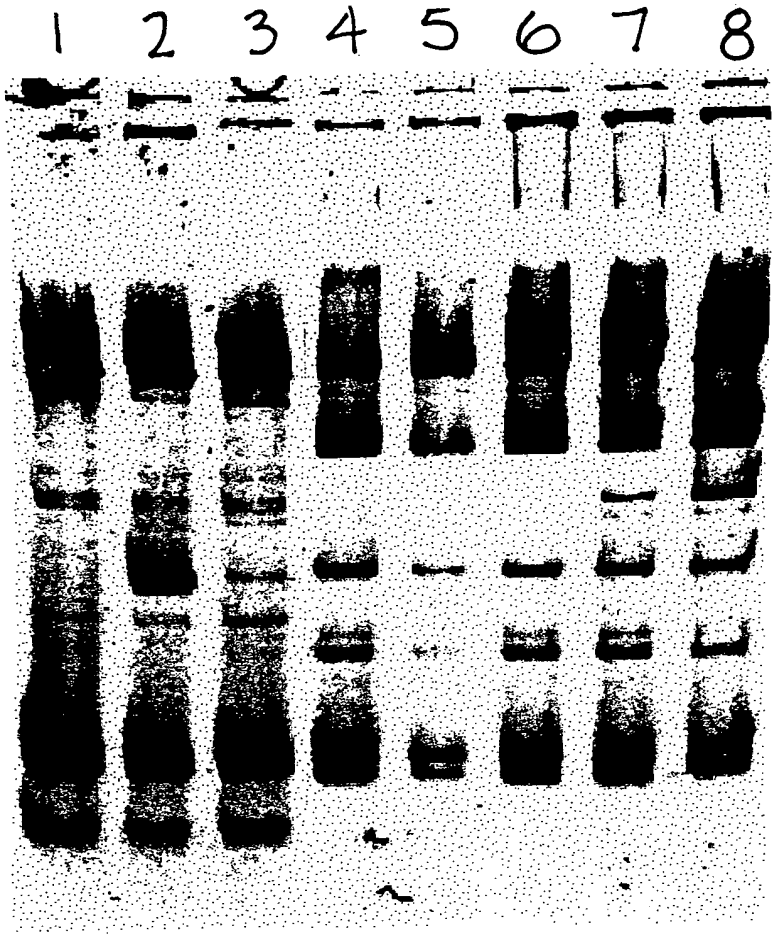
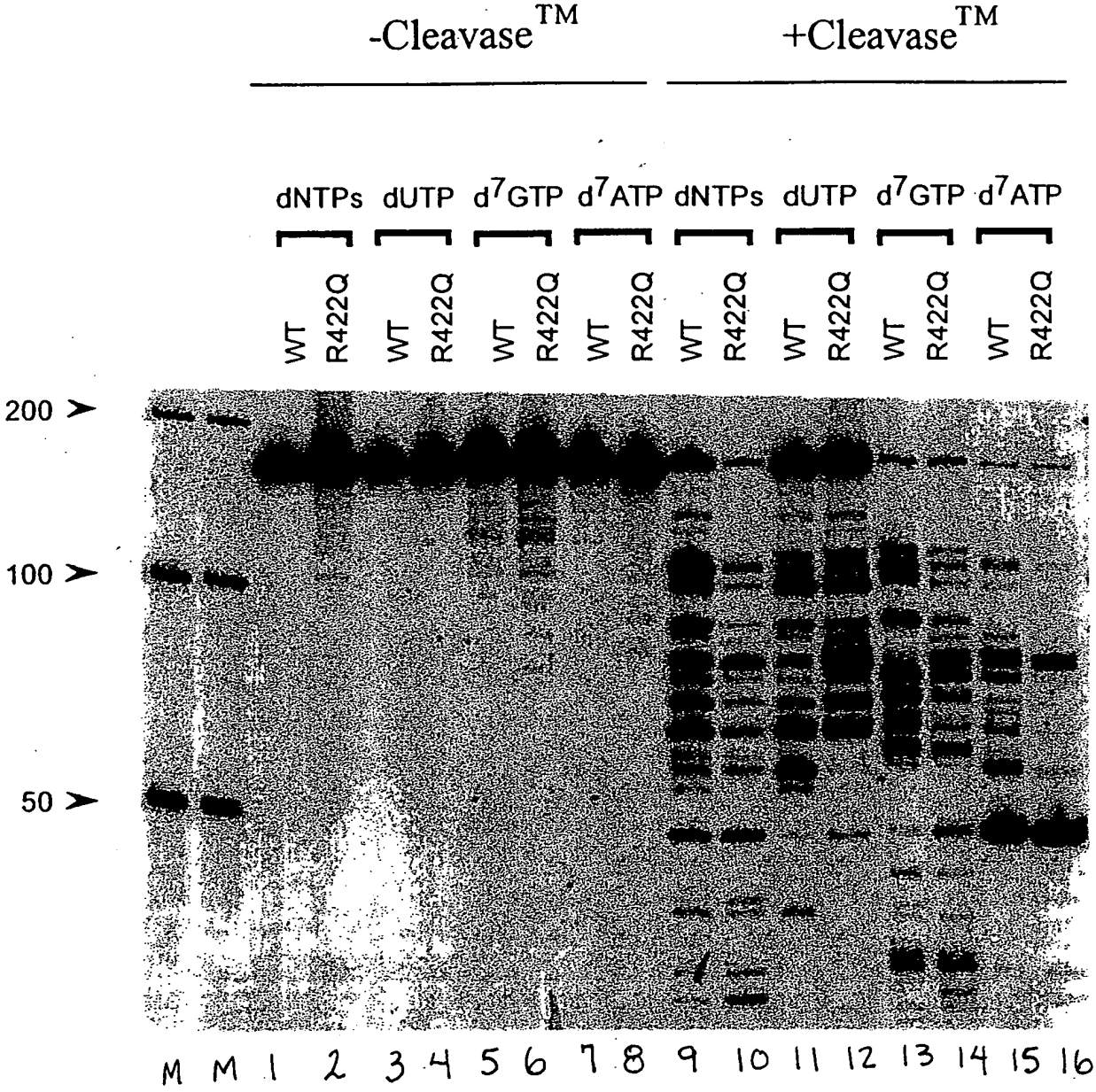


FIGURE 94


-Cleavase™
+Cleavase™
dNTPs
dUTP
d7GTP
d7ATP
dNTPs
dUTP
d7GTP
d7ATP
WT
R422Q
200
100
50
M M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16